Vol. 10 September, 1953 No. 2

JOURNAL OF THE FACULTY OF AGRICULTURE KYUSHU UNIVERSITY

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PUBLISHED BY THE FACULTY
FUKUOKA
1953

Journal of the Faculty of Agriculture, Kyūshū University, Vol. 10, No. 2
September 30, 1953

CYTOGENETICAL STUDIES ON THE STERILE WILD SENNA (*CASSIA TORA* L.) PRODUCED BY THE ATOMIC BOMB EXPLOSION

IV. On the gigas mutants segregated from the asynaptic wild senna (*Cassia Tora* L.)

TAIRA KATAYAMA

I. INTRODUCTION

In 1949 Nagamatsu[10] reported briefly on a sterile wild senna, *Cassia Tora* L., produced after the atomic bomb explosion in Nagasaki in 1945. Nagamatsu maintained that the sterility had been caused by an asynaptic characteristic which was inherited as simple Mendelian recessive. This fact was confirmed later by the author through more detailed cytological and genetical observations.[7,8]

Due to the failure of bivalent formation, the chromosome behaviour of the asynaptic plant during meiosis was very irregular. This phenomenon resulted in a high percentage of abortive pollen grains. Such irregular meiotic chromosome behaviour, which usually causes high sterility, may enhance the probability of chromosomal mutations, such as, haploid, triploid, heteroploid, etc.

In 1949 the author discovered two gigas type plants (No. 21–8 and No. 65–1) among the offspring of asynaptic wild senna. These two gigas plants were very similar morphologically, and had one extra chromosome each. The morphological aspects and chromosome behaviour in the pollen mother cells of these gigas plants and their offspring (No. 21–8–1 and No. 21–8–2) will be described in this paper.

II. Material

The materials used in this study were as follows: two gigas type plants (No. 21–8 and No. 65–1), and two progenies (No. 21–8–1 and No. 21–8–2). The former were discovered among the offspring of wild senna which have already been reported as asynaptic plants,[8,10,12] while the latter were produced by the self-pollination of plant No. 21–8.

The pedigrees of these materials are as follows:

1946	1947	1948	1949
Culture No. 18	E. 18–23 →Pollen → normal.	E. 17–10 Pollen normal. → Percentage of asynaptic plants in Line No. 17 was 9.09.	E. 21–8 Pollen Abortive. All other plants in the Line E. 21 were normal.
	E. 18–82 →Pollen → normal.	E. 22–4 Percentage of → abortive pollen was 99.15. Percentage of asynaptic plants in Line No. 22 was 29.03.	E. 65–1 Pollen abortive. Line E. 65 contained only one plant.

III. Morphological Characteristics (No. 21–8 and No. 65–1)

As stated elsewhere,[7] the asynaptic sterile plant of the wild senna is not distinguishable from the normal one morphologically. They can be discriminated only by the high sterility of the asynaptic plant at their fruit-bearing time. The two gigas mutants described here are distinctly different from both the normal and the asynaptic wild senna in such characteristics as gigas type, delayed flowering time, and high sterility (Table 1 and Figs. 1–4).

As shown in Table 1, the normal plants started to flower about July 25, while the gigas mutants produced buds about September 10 and their first flowers on September 20. The normal adult plant was approximately 105 cm. high, while the gigas

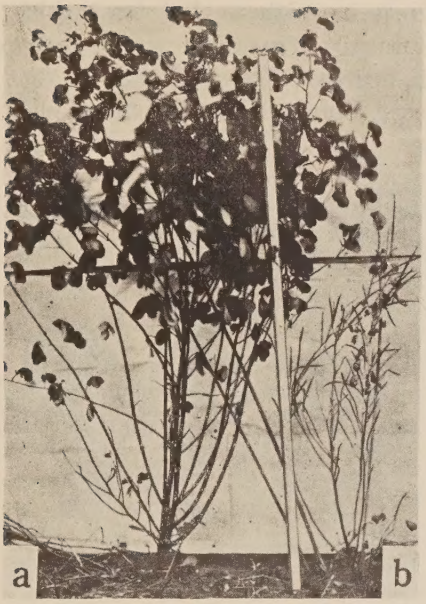


Fig. 1. Normal and gigas plants (trisomic plant) of *Cassia Tora* L.
a. Gigas plant (No. 21-8). b. Normal plant.

Table 1. Measurements on the normal and the gigas plants and their offspring.

	Normal plant	Gigas mutants		Offspring of No. 21-8	
		21-8	65-1	21-8-1	21-8-2
Date of the 1st flower	July 25	Sept. 20	Sept. 20	July 21	July 4
Height (cm)	104,9	180,0	178,0	160,0	120,0
No. of branches per plant	15,51	99,0	96,0	50,0	101,0
Stem diameter (cm)	1,10	2,27	2,07	—	—
No. of placentas per plant	30,47	30,60	31,50	—	30,39
No. of seeds per pod	10,08	2,67	0	2,03	11,02
Length of rachis (cm)	5,79	8,35	7,68	7,5	6,8
Length of 1st leaflet (cm)	3,22	4,72	4,63	4,5	4,3
Width of 1st leaflet (cm)	2,14	3,15	3,15	2,7	3,2
Length of 2nd leaflet (cm)	4,14	6,18	6,08	5,8	5,5
Width of 2nd leaflet (cm)	2,61	3,78	3,71	3,5	3,8
Length of 3rd leaflet (cm)	4,50	7,03	6,95	6,5	6,8
Width of 3rd leaflet (cm)	2,71	4,08	3,84	3,4	4,2
Length of guard cells	7,03	6,84	6,94	7,01	7,04
% of sterile pollen grains	23,75	89,35	86,42	90,13	44,21

mutants were nearly as high as 180 cm., which is almost twice as high as the former. The diameter of the main stem of the mutant was also nearly twice as large as that of the normal. The total number of branches on the normal plant was 15.5, while those of the gigas mutants numbered about 100. The rachis of the gigas mutants were longer than those of the normal by about 2.0–2.5 cm. The length and width of each leaflet were also greater

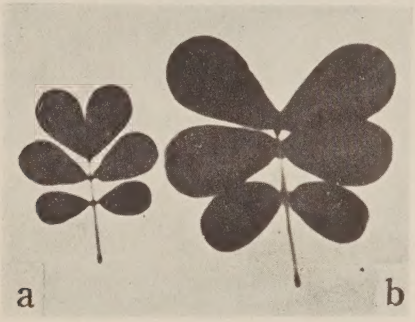


Fig. 2. Compound leaf.
a. Normal plant. b. Gigas plant.

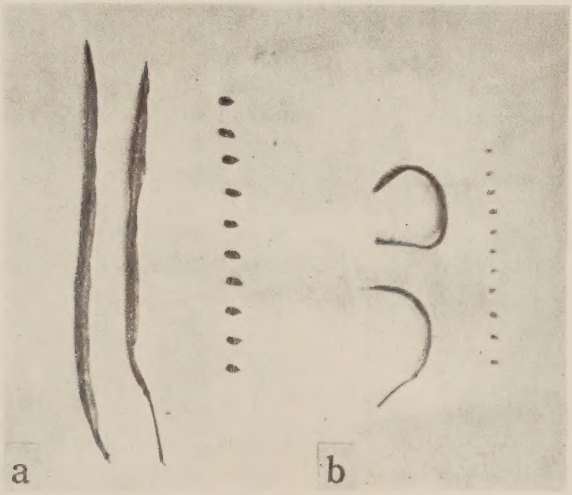


Fig. 3. Pods and seeds.
a. Normal plant. b. Gigas plant.

by about 1–2 cm., respectively. Another distinct difference was also observed in the size of the flower-vase. However, no difference was noticed in the length of the guard cell and in the number of placentae per legume between the two types.

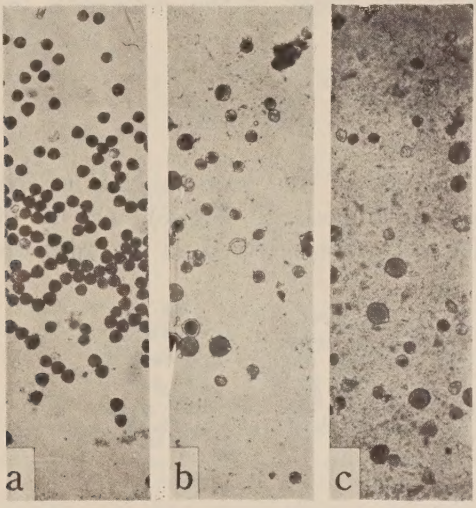


Fig. 4. Pollen grains (× 54).
a. Normal plant.
b. Gigas plant (No. 21–8).
c. „ „ (No. 65–1).

IV. Methods

The root tips were fixed with Navashin's solution and stained with Heidenhain's iron-alum-haematoxylin.

The pollen mother cells were fixed with acetic alcohol (alcohol 3 : glacial acetic acid 1) for about one hour, and then stained with Heidenhain's iron-alum-haematoxylin and Newton's gentian violet. A good result was obtained from the latter.

Sections of the root tips were sliced as thick as 7 to 8μ, and those of the pollen mother cells from 18 to 20μ.

Pollen tetrads and pollen sterility were investigated by the smear method with iron acto-carmine.

V. Cytological Observations
(No. 21–8 and No. 65–1)

Since the behaviour of the chromosomes in the two gigas mutants, discovered in 1949 were similar to each other, the author will describe only the result of the cytological observations on plant No. 21–8.

Root Tips

As stated elsewhere,[8] the root tip cells of the normal wild senna contained 26 chromosomes (Fig. 5). On the other hand, 27 chromosomes (2n+1) were observed in the root tip of the gigas

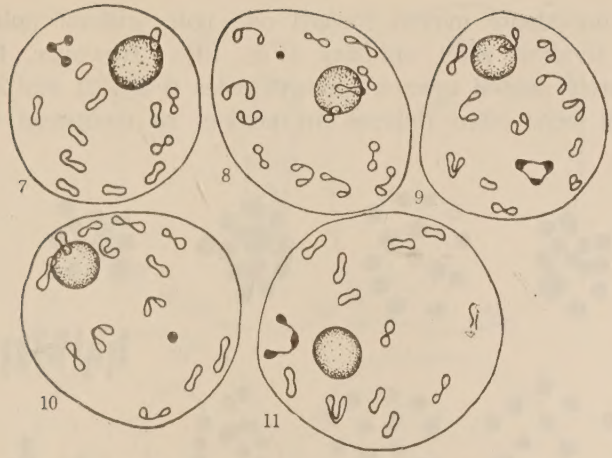
Figs. 5-6. Somatic chromosomes (ca. ×2250).
5. Normal plant (2n=26). 6. Gigas plant (2n=27).

mutants (Fig. 6). This fact indicated that the gigas mutants were so-called "trisomics", with one extra chromosome. It was impossible, however to distinguish the extra chromosome morphologically.

Pollen Mother Cells

(Heterotypic division)

In the diakinetic nucleus of the pollen mother cells, chromosome conjugations of $1_{III}+12_{II}$ were counted in about 59% (Fig. 7), and $13_{II}+1_{I}$ in about 41% (Fig. 8; Table 2). At the first metaphase, $1_{III}+12_{II}$ were observed in half of the pollen mother cells (Figs. 14 and 19). $13_{II}+1_{I}$ were next to the $1_{III}+12_{II}$ chromosomes in number (Figs. 13 and 20). However, the $1_{IV}+1_{III}+10_{II}$ (Fig. 12) were rarely found (Table 2). In these cases, univalents, bivalents, and trivalents could be distinguished from one another



Figs. 7-11. Diakinesis. 7-8. No. 21-8, showing $1_{III}+12_{II}$ and $13_{II}+1_{I}$ respectively. 9-10. No. 65-1, showing $1_{III}+12_{II}$ and $13_{II}+1_{I}$ respectively. 11. No. 21-8-1, showing $1_{III}+12_{II}$ (ca. ×2250).

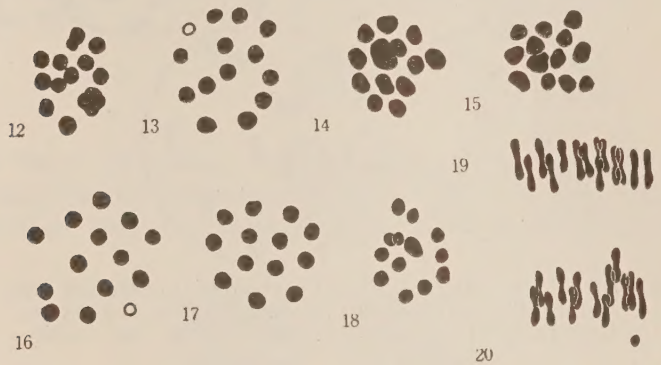
Table 2. Frequencies of PMCs with various degrees of chromosome association at diakinesis and M-I.

Culture No.	Stage	Frequencies of chromosome association			
		$1_{IV}+1_{III}+10_{II}$	$1_{III}+12_{II}$	$13_{II}+1_{I}$	13_{II}
Normal plant	Diakinesis	—	—	—	20
	M-I	—	—	—	23
21-8					
	Diakinesis	—	26	18	—
	M-I	1	22	18	—
65-1					
	Diakinesis	—	9	8	—
	M-I	—	20	17	—
21-8-1					
	Diakinesis	—	22	15	—
	M-I	—	13	11	—
21-8-2					
	Diakinesis	—	—	—	10
	M-I	—	—	—	13

by shape, location, and degree of stain, while the tetravalents were determined by the shape and size.

At the first anaphase, the bivalents were normally disjoint, the halves moving toward each pole. At the first telophase, they were included in the daughter nuclei. Trivalents are usually disjoint; two chromosomes moving to one pole and the other moving toward the opposite pole. In most cases, it was observed

that the univalents moved toward one pole without splitting, or without dividing after splitting (Fig. 21). However, the split halves rarely passed toward opposite poles (Figs. 21 and 23). The univalents were often delayed in moving, as compared with the



Figs. 12-18. Polar views of M-I. 12-14. No. 21-8, showing $1_{IV}+1_{III}+10_{II}$, $13_{II}+1_{I}$ and $1_{III}+12_{II}$ respectively. 15-16. No. 65-1, showing $1_{III}+12_{II}$ and $13_{II}+1_{I}$ respectively. 17. No. 21-8-2, showing 13_{II}. 18. No. 21-8-1, showing $1_{III}+12_{II}$ (ca. ×2250).

Figs. 19-20. Side views of M-I. 19. No. 21-8, showing $1_{III}+12_{II}$. 20. No. 65-1, showing $13_{II}+1_{I}$ (ca. ×2250).

bivalents (Figs. 21 and 23), and consequently at interkinesis, some micronuclei besides the two daughter nuclei were frequently formed (Fig. 23). The polyvalent chromosomes, such as the tetravalent, were rarely found at M-I (Fig. 12).

(Homeotypic division)

Usually 13 (Fig. 27) or 14 (Fig. 28), and occasionaly 12 chromosomes (Fig. 26) could be counted during the second metaphase. Some lagging chromosomes were also observed at the second anaphase. Occasionally, at cytokinesis, these laggards formed the micronuclei in addition to four daughter nuclei.

(Tetrads and pollens)

At the stage, corresponding to the pollen tetrads, the irregular sporads consisted of monads to hexads. In No. 21-8 and No. 65-1, the number of tetrads was equivalent to 70 and 77%, respectively, of all sporads; 67 and 47%, respectively, of the tetrads were distinctly abnormal in size or shape (Table 3).

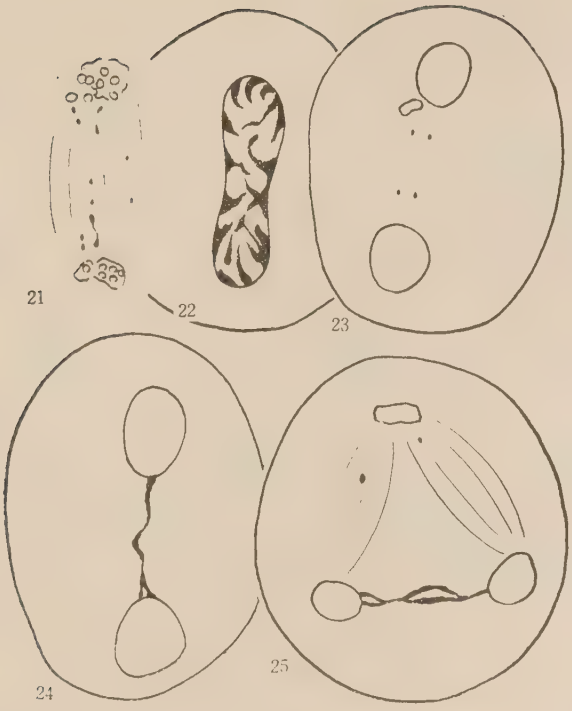
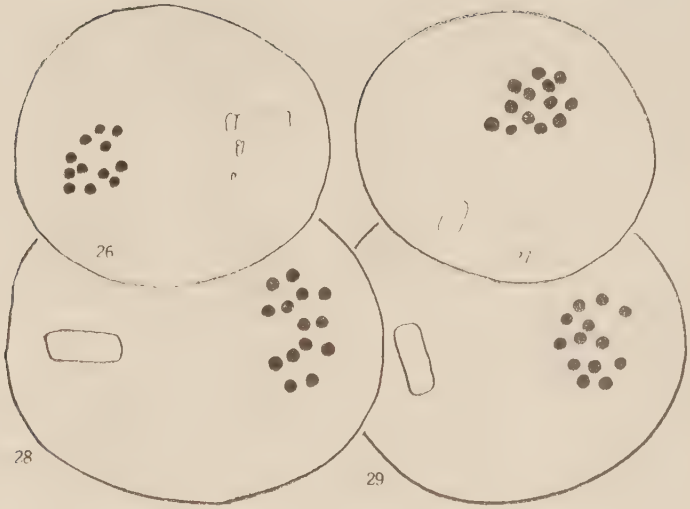


Fig. 21. A-I of No. 21-8, showing lagging chromosomes.
Fig. 22. Regression in No. 21-8.
Fig. 23. No. 21-8, showing a micronucleus and some laggards.
Fig. 24. No. 21-8, showing a chromosomal bridge at interkinetic stage.
Fig. 25. No. 21-8, showing tripolarity (ca. ×2250).

According to the experiment conducted at the end of October in 1949, if the pollen, not stained with acetocarmine, was presumed as the abortive pollen, than these gigas mutants showed sterility of pollen as high as 89 and 86% respectively, while the pollen sterilities of the normal plants showed about 24% (Fig. 4a, b, and c; Table 1).

In the normal plant the size of the pollen grains was comparatively equal, ranging from 3.0 to 4.5, (where the figures refer to the graduations on the micrometer scale), with the mode at 3.75. On the other hand, in the gigas mutants the size varied, and ranged from 3.0 to 6.5, with the mode between 4.25 and 5.25 (Fig. 4a, b, c; Table 4).



Figs. 26-28. M-II of No. 21-8, showing 12, 13 and 14 chromosomes respectively.
Fig. 29. M-II of No. 21-8-1, showing 13 chromosomes (ca. ×2250).

Table 3. Frequencies of various sporads in tetrad stage.

Culture No.	Monads	Diads	Triads	Tetrads		Pentads	Hexads	Total
				Normal	Abnormal			
Normal plant	—	—	—	99	—	1	—	100
21-8	4	12	47	118	148	24	2	355
65-1	3	32	41	240	77	19	2	414
21-1-1	5	16	55	132	161	33	4	406
21-1-2	—	—	5	78	28	9	—	120

In spite of numerous self-pollinations, the gigas mutants always showed high sterility. Thus, the assumption was made that most of their pollen was abortive.

(Other Irregularities)

Other types of irregularities such as chromosome bridges (Figs. 24 and 25), tripolarity (Fig. 25), and regression (Fig. 22), were rarely observed during the first anaphase and telophase.

Table 4. Size distributions of pollen grains of the normal and the trisomic plants and their offspring.

1 unit=3.3	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	Total	$\bar{x}$	s^2
Normal plant		—	109	73	—	—	—	—	—	—	182	3.95	0.0604
21-8		8	21	54	35	95	14	2	—	—	229	4.77	0.4152
65-1		18	30	58	58	60	4	1	—	1	230	4.55	0.4690
21-1-1		7	26	51	48	89	9	1	—	—	231	4.72	0.3817
21-1-2		71	170	58	—	—	—	—	—	—	299	3.73	0.1077

VI. The Offspring of No. 21-8.

In 1950 11 seeds, obtained from the self-pollination of plant No. 21-8 in 1949, were sowed in Wagner's pot (1/20.000 Tan; one acre equals 4.0806 Tan). Only three of these seeds germinated, and the two plants grown were put into a sun-glass room until flowering time. The pots were then moved from the room.

Morphological Observations

(No. 21-8-1)

Unfortunately, this plant died at the beginning of September. This plant was very similar to its parent (No. 21-8), except for the discrepancy in the beginning of flowering time and in number of branches (Table 1). The flowering time of this plant was earlier than that of the parent. This phenomenon may have resulted because of its environment in the sun-glass room during the earlier stage. In addition, death in the early stage may have resulted in a shorter stem and fewer branches.

(No. 21-8-2)

The morphological characteristics of this plant were very similar to those of the normal one, although the former showed more abortive pollen grains (Table 1). The reason for the premature flowering of this plant also seems to be the same as that stated in the preceding paragraph; and the multiplicity in the number of branches may be due to the fact that it was provided with sufficient space for growth.

Cytological Observations

(No. 21–8–1)

The somatic chromosome number of No. 21–8–1 was counted as 27 (2n+1).

At diakinesis $1_{III}+12_{II}$ and $13_{II}+1_{I}$ were often perceived (Fig. 11). Although the number of cases observed were not so many during the first metaphase, $1_{III}+12_{II}$ (Fig. 18) and $13_{II}+1_{I}$ were shown as a result. In most cases, 13 chromosomes were counted in one plate during the second metaphase (Fig. 29), although 12 and 14 chromosomes were observed. The behaviour of the chromosomes during the first anaphase, interkinesis, and second metaphase, as well as the general appearance of the tetrads and pollen were similar to those of the parent (No. 21–8).

(No. 21–8–2)

In the root tips of plant No. 21–8–2, 26 chromosomes were counted. At diakinesis and the first metaphase 13_{II} were observed (Fig. 17); at the second metaphase 13 chromosomes were counted. The behaviour of the chromosomes appeared to be similar to that of the normal ones.

VII. Discussion

In *Datura*,[3] *Nicotiana*,[5,14] *Oryzae*,[6,11] and *Oenothera*,[2] trisomic plants were artificially produced by exposure to X-rays or radium.

In wild senna, it seems that the gigas plants, having one extra chromosome were produced as the result of non-disjunction by irregular distribution of univalents to the offspring of the asynaptic plants. The above plants were brought about by gene mutation, produced artificially by the atomic bomb explosion. This has the same effect on these plants as X-rays and radium.

In *Datura*, when self-pollinated, the trisomic character was mainly transmitted at the rate of about 25% from the ovules. The "Globe" character of the trisomic "Globe" type was inherited by 3% through the pollen, while 25% through the ovules.[1]

On the other hand, the line which produced trisomic plants only was discovered among the offspring of "dwarf mutant plant × normal plant" in *Oenothera*[4] and of "*T. polonicum* × *T. spelta*."[15]

It is a characteristic of the inheritance in trisomics that the

ratio of segregation depends not upon the chance, but upon the result of the control of the various conditions; namely, in most cases, the production rate of trisomic plants is usually less than that of normal ones.

Only two seeds germinated out of 12 seeds obtained from the self-pollination of plant No. 21–8. One of them (No. 21–8–1) is very similar to the parent (No. 21–8) in morphological and cytological character, while the other resembles the normal one, as stated previously. To his regret, the author could not investigate the inheritance of the former (No. 21 8–1) because of its death during the flowering time. In the following generation, no gigas plant was produced from the latter (No. 21–8–2), so that the inheritance of the trisomic plant can not be assured in this paper.

Usually trisomic types were characterized by decreasing the growing vigour, according to one extra chromosome.[9,10] But these two gigas plants and one offspring of plant No. 21–8, having one extra chromosome respectively, increased the size of various parts (Figs. 1 and 2; Table 1).

VIII. Summary

1). Morphological investigations and cytological studies were performed on the two gigas mutants, produced in the offspring of the asynaptic wild senna in 1949. Cytological studies have determined that these gigas mutants are trisomic plants with 27 chromosomes (2n+1).

2). These gigas mutants were characterized by gigas type, delay of flowering time and high sterility.

3). In the pollen mother cells, $1_{III}+12_{II}$ and $13_{II}+1_{I}$ were observed in most cases; and $1_{IV}+1_{III}+10_{II}$ in fewer cases.

4). At, and after the first anaphase, various irregularities could be observed. Lagging chromosomes and irregular sporads were often noticed, but bridge, regression, and tripolarity were rarely seen.

5). In the cytological examination of two offspring, which were produced from the self-pollination of plant No. 21 8, observations showed that one resembled the parent, while the other resembled the normal plant.

IX. Acknowledgments

I wish to acknowledge my deep indebtedness to Dr. T. Morinaga and to Assistant Prof. T. Nagamatsu for their kind guidance and encouragement during the progress of this work. I am also exceedingly grateful for the grant from the Science Research Fund of the Ministry of Education for this study.

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Journal of the Faculty of Agriculture, Kyūshū University, Vol. 10, No. 2
September 30, 1951

SPHECOIDEA OF MICRONESIA. 4*
REVISION OF THE GENUS *PISON* SPINOLA. PART 1
(Hymenoptera : Sphecidae)

KEIZÔ YASUMATSU

It was Georg Semper (1905) who first reported the occurrence of the *Pison*-species† in Micronesia. Since the publication of my first paper (1937), in which I recorded four species of the genus, a good number of material have been accumulated owing to the elaborate expeditions to the main islands of Micronesia by Professor Teiso Esaki and his collaborators. During the course of my taxonomic study of the material, it became necessary to publish a revision of the genus *Pison* in Micronesia. Meanwhile Krombein (1949, 1950) published an extensive works on the Micronesian wasps and bees which were collected by some American entomologists chiefly during the years 1945–1949. In his first work Krombein recorded eleven species of *Pison*, of which three were new to science. The present paper is made as a supplement to Krombein's works together with a distributive study of the genus in the Pacific Islands and gives some new morphological data in the consideration of the affinity among the species.

In the first I desire to express my sincere gratitude to Professor T. Esaki for his very kind guidance in many ways rendered in the course of the present study. I am deeply indebted to Mr. Karl V. Krombein, of the U. S. National Museum, for the gift of specimens of two species which were not collected by us.

* Results of Professor T. Esaki's Micronesia Expeditions 1936-1940, No. 81.

† The species was recorded by Semper under the name *Pison rugosus* Smith, but, so far as my knowledge goes, this species is nothing but *Pison punctifrons* Shuckard.

Enumeration of the Species and their Distribution in the Pacific Islands

So far as I am aware, the following twenty species of *Pison* have been known in the Pacific Islands.

1. *Pison argentatum* Shuckard ...Westward from Madagascar eastward to Hawaii, and southward to Micronesia.
2. *Pison collare* Kohl.....New Britain.
3. *Pison esakii* Yasumatsu .....Marianna Islands.
4. *Pison glabrum* Kohl .....Samoa.
5. *Pison hospes* Smith .....Westward from Malaya eastward to Hawaii, and southward to Marquesas Islands.
6. *Pison ignavum* Turner.....Palau Islands, Australia, New Caledonia, Fiji, Samoa, Society Islands, Marquesas Islands.
7. *Pison impunctatum* Turner.....New Guinea, Society Islands, Marquesas Islands.
8. *Pison insulare* Smith .....New Hebrides, Banks Islands, Hawaii.
9. *Pison iridipenne* Smith.....Marianna Islands, Palau Islands, Caroline Islands, Marshall Islands, Australia, Fiji, Samoa, Hawaii, Society Islands, Tuamotu Archipelago.
10. *Pison korrorense* Yasumatsu ...Palau Islands.
11. *Pison mariannense* Yasumatsu ..Marianna Islands.
12. *Pison nigellum* Krombein .....Caroline Islands.
13. *Pison novocaledonica* Krombein ..New Caledonia.
14. *Pison oakleyi* Krombein .....Marianna Islands.
15. *Pison ponape* Krombein .....Caroline Islands.
16. *Pison punctifrons* Shuckard ...Westward from N. W. India eastward to Marshall Islands.
17. *Pison strictifrons* Vachal.....New Caledonia.

18. *Pison tahitense* Saussure.....Marshall Islands, Ellice Islands, Australia (?), Fiji, Samoa, Tonga, Tahiti, Society Islands, Marquesas Islands.

19. *Pison tosawai* Yasumatsu .....Bonin Islands.

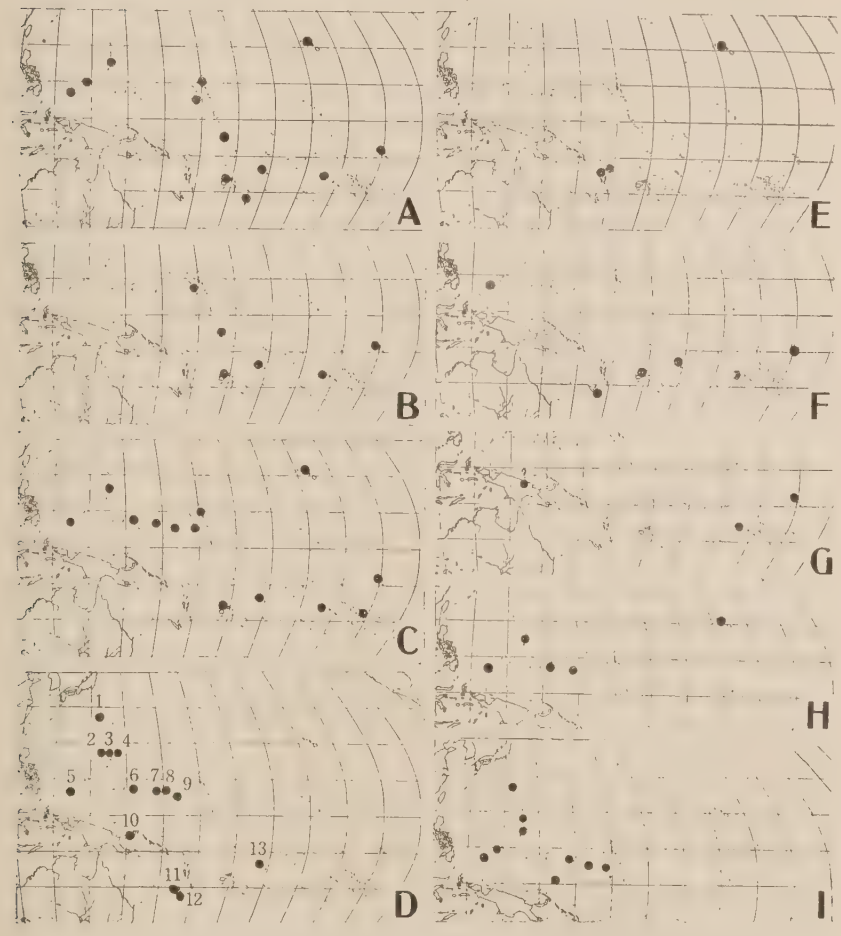

Fig. 1. Distribution of *Pison*-species in the Pacific Islands.
A: *hospes*, B: *tahitense*, C: *iridipenne*, D: Endemic species (1–*tosawai*, 2–*esakii*, 3–*mariannense*, 4–*oakleyi*, 5–*korrorense*, 6–*trukense*, 7–*ponape*, 8–*ponape*, 9–*nigellum*, 10–*collare*, 11–*novocaledonica*, 12–*strictifrons*, 13–*glabrum*), E: *insulare*, F: *ignavum*, G: *impunctatum*, H: *argentatum*, I: *punctifrons*.

20. *Pison trukense* Yasumatsu .....Caroline Islands.

From the zoogeographical point of view the twenty *Pison*-species listed above may be classified into the following groups of different origins.

Group I. *Pison* of the Indo-Malayian origin (1 species) .... *punctifrons.*

Group II. *Pison* of the Polynesian origin (10 species)

Subgroup I. *Pison* of the Micronesian origin (7 species)

Section A. *Pison* of the Bonin origin .....*tosawai.*

Section B. *Pison* of the Marianna origin .... *esakii, oakleyi, mariannense.*

Section C. *Pison* of the Caroline origin .... *nigellum, ponape, trukense.*

Subgroup 2. *Pison* of the Polynesian (s. str.) origin (3 species) ..... *glabrum, tahitense, impunctatum.*

Group III. *Pison* of the Melanesian origin (6 species)

Subgroup 1. *Pison* of the Palau origin .....*korrorense.*

Subgroup 2. *Pison* of the southern islands origin ....*strictifrons, novocaledonica, collare, insulare, ignavum* (?).

Group IV. *Pison* of the uncertain origin (3 species) .... *argentatum, hospes, iridipenne.*

Thus it may not be difficult to derive several peculiaristic characters concerning the *Pison*-fauna of the Pacific Islands from the above classification. There is no relation between the *Pison*-fauna of the Pacific Islands and those of the Australasian Region. This fact may well support the theory of Professor Esaki (1942, 1950), namely "the whole Pacific Islands except those closely situated to Australia and New Zealand and to the American Continents, should belong to the Oriental Region instead of to the Australian Region, so far as the insects are concerned." Of the twenty species 50 per cent are of the Polynesian origin and 30 per cent of the Melanesian one. Micronesia has the richest endemic fauna of the Pacific Islands. A very interesting fact is that in Micronesia there is found not a single endemic form which occurs throughout on all of the islands or even on two different island-groups. For example, *esakii*, *mariannense* and

oakleyi occur only in the Marianna Islands but in neither the Caroline nor the Palau Islands. *Korrorense* is found only in the Palau Islands but not in Yap Island which is situated close to the former. It is rather curious that not a single endemic *Pison*-species was ever found in Yap Island and also in Hawaii, where there have been differentiated many endemic species among other groups of insects. Next it is an important evidence that there is not a single species which is found both in Micronesia and Hawaii but not elsowhere. This phenomenon may be explained by the habitat of the Micronesian endemic species, i. e. *esakii, oakleyi, mariannense, ponape, nigellum* or *trukense*. These species are all inhabitants of the native forests presumably making their nests in the ground. The similar relation may exist in such species as *collare, glabrum, korrorense, novocaledonica, strictifrons* and *tosawai*. In 1911 R. E. Turner suggested the possibility of transportation of the *Pison*-species from one district to another writing as follows:—"Such of the species as having been observed build mud nest in holes in wood, key-holes, or similar situations, stocking their nests with small spiders, which paralysed by stinging. Owing to these habits the species are easily transported on ships, giving rise to a considerable extension of range in several species, such as *P. spinolae* and *P. argentatum*." The following species may belong to this category in relation to their mode of distribution: *argentatum, hospes, ignavum, impunctatum, insulare, iridipenne, punctifrons* and *tahitense*. Another peculiarity of the *Pison*-fauna in the Pacific Islands which must not be overlooked is that all are the black species and not a single representative of the light coloured species occurs in this area.

The distribution of the Micronesian species is summarised in detail as follows:

1. Species occuring in the Marianna Islands—*argentatum, esakii, hospes, iridipenne, mariannense, oakleyi, punctifrons* (7 species).
2. Species occuring in the Palau Islands—*argentatum, hospes, ignavum, iridipenne, korrorense, punctifrons* (6 species).
3. Species occuring in the Caroline Islands—*argentatum, iridipenne, nigellum, ponape, punctifrons, trukense* (6 species).
4. Species occuring in the Marshall Islands—*hospes, iridipenne, punctifrons, tahitense* (4 species).

Time of Occurrence of Micronesian *Pison*-Species

As regards the time of occurrence of Micronesian *Pison*-species, it appears that almost all the species may occur throughout the year as given in the following table.

Table 1. Collecting data of Micronesian *Pison*-species.

Species	Month of capture of Micronesian *Pison*-species											
	i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii
argentatum	●	●	●	●	●	●	●	●	●	●	●	●
esakii		●	●		●	●	●		●			
hospes		●	●	●	●		●	●	●			●
ignavum		●	●	●								
iridipenne	●	●				●	●	●			●	●
korrorense		●	●					●				
mariannense					●						●	
nigellum	●	●	●					●	●		●	
oakleyi				●	●	●					●	●
ponape	●	●	●				●	●			●	●
punctifrons	●	●	●	●	●	●	●	●			●	●
tahitense							●	●				
trukense				●								

Systematic List of the Species of *Pison* in Micronesia

Pison argentatum Shuckard, 1837

Synonym: *P. fuscipalpis* Cameron, 1901.

Bridwell, Proc. Hawn. Ent. Soc., 4: 123 (Hawaii); Swezey, B. P. Bishop Mus. Bull., 172: 185 (Guam); Krombein, 1949, Proc. Hawn. Ent. Soc., 13: 403 (Mariana Islands—Guam; Caroline Islands—Truk, Ponape); Krombein, 1950, Proc. Hawn. Ent. Soc., 14: 139 (Yap).

Habitat in Micronesia: Marianna Islands—Saipan, Tinian, Rota, Guam; Palau Islands—Korror; Caroline Islands—Yap, Truk, Ponape.

Distribution: Madagascar, Mauritius, Tenasserim, Burma, Malaya, Borneo, Philippines, Marianna Islands, Palau Islands, Caroline Islands and Hawaii.

Specimens examined: 1♀, 10. xi. 1937, Garapan, Saipan,

Marianna Islands, Esaki leg.; 2♂♂ 1♀, 7. vii. 1939, Garapan, Saipan, Esaki leg.; 1♂, 4. v. 1940, Garapan–Sadog Tasi, Saipan, Yasumatsu et Yoshimura leg.; 2♂♂ 1♀, 7. v. 1940, Donni–Sadog Tasi, Saipan, Yasumatsu et Yoshimura leg.; 3♂♂ 2♀♀, 3. xi. 1937, Marupo–Hagoi, Tinian, Marianna Islands, Esaki leg.; 1♀, 5. xi. 1937, Tetêto–Tatâcho–Soñgsoñg, Rota, Marianna Islands, Esaki leg.; 1♀, 10. v. 1939, Arabaketsu, Korror, Palau Islands, S. Miyake leg.; 1♀, 25. viii. 1940, Arabaketsu, Korror, Nagasawa leg.; 1♀, 10. ix. 1940, Arabaketsu, Korror, Nagasawa leg.; 1♀, 17. i. 1938, Kolonia, Ponape, Caroline Islands, Esaki leg.; 1♀, 24. vii. 1939, Kolonia—Jokaji, Ponape, Esaki leg.

Pison esakii Yasumatsu, 1937

Yasumatsu, 1937, Mushi, 9: 129 (Marianna Islands); Yasumatsu, 1939, Festschr. 60. Geburtst. E. Strand, 5: 83; Krombein, Proc. Hawn. Ent. Soc., 13: 401 (Mariana Islands). *P.* sp. Fullaway, 1913, Proc. Hawn. Ent. Soc., 2: 283 (Guam); Swezey, 1942, B. P. Bishop Mus. Bull., 172: 185 (Guam).

Habitat in Micronesia: Marianna Islands–Saipan, Tinian, Rota, Guam.

Distribution: Marianna Islands.

Specimens examined: 1♀, 11. iii. 1938, Tapôcho—Garapan, Saipan, Marianna Islands, Esaki leg.; 1♀, 1. vii. 1939, Garapan, Saipan, Esaki leg.; 12♀♀, 3. v. 1940, Matansha–Calabera, Saipan, Yasumatsu et Yoshimura leg.; 1♀, 6. v. 1940, Tapôcho, Saipan, Yasumatsu et Yoshimura leg.; 4♀♀, 12. v. 1940, Fanagam, Saipan, Yasumatsu et Yoshimura leg.; 1♀, 8. ii. 1936, Tatâcho–Soñgsoñg, Rota, Marianna Islands, Esaki leg.; 1♀, 5. xi. 1937, Tetêto—Tatâcho—Soñgsoñg, Rota, Esaki leg.

Pison hospes Smith, 1879

Smith, 1879, Jour. Linn. Soc. Zool., 14: 676 (Hawaii); Bridwell, Proc. Hawn. Ent. Soc., 4: 123 (Hawaii); Perkins et Cheesman, 1928, Insects of Samoa, 5, fasc. 1: 27 (Samoa); Cheesman, 1928, Ann. Mag. Nat. Hist., ser. 10, 1: 175 (Marquesas, Society Islands); Williams, 1932, B. P. Bishop Mus. Bull., 98: 151 (Marquesas); Krauss, 1944, Proc. Hawn. Ent. Soc., 12: 93 (Molokai); Williams, 1947, Occasional Pap., B. P. Bishop Mus., 18: 331; Krombein, 1949, Proc. Hawn. Ent. Soc., 13: 404 (Marshall Islands, Palau Islands); Krombein, 1950, Proc. Hawn. Ent. Soc., 14: 139 (Bikini-Atoll). *P. fuscipennis* Yasumatsu (nec Smith), 1937, Mushi, 9: 131 (Palau Islands); Yasumatsu, 1939, Festschr. 60. Geburtst. E. Strand, 5: 83.

Habitat in Micronesia: Marianna Islands–Saipan; Palau

Islands—Babeldaob, Korror, Peliliu; Caroline Islands—Yap; Marshall Islands—Ailinglapalap-Atoll, Jaluit-Atoll, Wotje-Atoll.

Distribution: Cocos-Keeling Island, Singapore, Marianna Islands, Palau Islands, Caroline Islands, Marshall Islands, Fiji, Samoa, Tonga Islands, Ellice Islands, Society Islands, Marquesas Islands and Hawaii.

Specimens examined: 1♂, 5. v. 1940, Garapan—Sadog Tasi, Saipan, Marianna Islands, Yasumatsu et Yoshimura leg.; 1♀, 11. v. 1940, Chalan Canoa, Saipan, Yasumatsu et Yoshimura leg.; 1♀, 12. v. 1940, Fanagam, Saipan, Yasumatsu et Yoshimura leg.; 1♂, 7. ix. 1939, Nif—Guilifez, Yap, Caroline Islands, Esaki leg.; 1♂ 1♀, 8. ix. 1939, Guilifez—Rul, Yap, Esaki leg.; 1♀, 9. ix. 1939, Rul, Yap, Esaki leg.; 1♀, 13. ix. 1939, Rul, Yap, Esaki leg.; 1♂ 1♀, 26. viii. 1946, Airek, Ailinglapalap-Atoll, Marshall Islands, H. K. Townes leg.; 1♂, 23. ii. 1936, Marukyoku, Babeldaob, Palau Islands, Esaki leg.; 1♀, 1. iii. 1936, Airai, Babeldaob, Esaki leg.; 1♀, 26. ii. 1936, Ngaraudo—Arukorum, Babeldaob, Esaki leg.; 1♂ 1♀, 7. ii. 1938, Ngardok—Ngarasumao, Babeldaob, Esaki leg.; 2♀♀, 11. ii. 1938, Ngardok—Ngarmisukan, Babeldaob, Esaki leg.; 1♂, 17. ii. 1936, Korror, Korror, Palau Islands, Esaki leg.; 1♀, 22. ii. 1936, Korror—Arabaketsu, Korror, Esaki leg.; 1♀, 22. xii. 1937, Arabaketsu, Korror, Murakami leg.; 1♂, 28. iv. 1939, Arabaketsu, Korror, Miyake leg.; 2♂♂, 9. viii. 1939, Korror, Korror, Esaki leg.; 2♀♀, viii. 1939, Korror, Korror, Esaki leg.; 1♀, 11. ix. 1940, Arabaketsu, Korror, Nagasawa leg.

Pison ignavum Turner, 1908

Turner, 1908, Proc. Zool. Soc. London: 511 (Australia); Williams, 1932, B. P. Bishop Mus. Bull., 98: 152 (Marquesas); Williams, 1945, Proc. Hawn. Ent. Soc., 12: 440 (New Caledonia); Williams, 1947, Occasional Pap., B. P. Bishop Mus., 18: 330 (Fiji); Krombein, 1949, Proc. Hawn. Ent. Soc., 13: 404 (Palau Islands). *P. argentatum ignavum* Turner, 1916, Proc. Zool. Soc. London: 601 (Australia, Fiji); Perkins et Cheesman, Insects of Samoa, 5, Ifasc. 1: 28 (Samoa); Cheesman, 1928, Ann. Mag. Nat. Hist., ser. 10, 1: 177 (Marquesas, Society Islands).

Habitat in Micronesia: Palau Islands—Korror.

Distribution: Palau Islands, Australia, New Caledonia, Fiji, Samoa, Society Islands and Marquesas Islands.

Specimens examined: 1♀, 11. iv. 1925, Papeiti, Tahiti, J. M. Clements leg.; 1♂, 2. ii. 1927, Papeiti, Tahiti, Clements leg.

Pison iridipenne Smith, 1879

Williams, 1932, B. P. Bishop Mus. Bull., 98 : 152 (Marquesas); Krauss, 1944, Proc. Hawn. Ent. Soc., 12 : 93 (Molokai); Williams, 1947, Occasional Pap., B. P. Bishop Mus., 18 : 331; Krombein, 1949, Proc. Hawn. Ent. Soc., 13 : 408 (Marshall Islands, Caroline Islands—Truk, Ponape, Palau Islands, Marianna Islands); Krombein, 1950, Proc. Hawn. Ent. Soc., 14 : 139, figs. 28, 35, 37 (Saipan). *P. iridipennis* Smith, 1879, Jour. Linn. Soc. Zool., 14 : 676 (Hawaii); Bridwell, Proc. Hawn. Ent. Soc., 4 : 123 (Hawaii); Perkins et Cheesman, Insects of Samoa, 5, fasc. 1 : 28 (Samoa); Cheesman, 1928, Ann. Mag. Nat. Hist., ser. 10, 1 : 176 (Marquesas, Society Islands).

Habitat in Micronesia: Marianna Islands—Tinian; Palau Islands—Peliliu; Caroline Islands—Truk, Ponape, Kusaie; Marshall Islands—Ailinglapalap-Atoll, Jaluit-Atoll, Wotje-Atoll.

Distribution: Marianna Islands, Caroline Islands, Palau Islands, Marshall Islands, Australia, Fiji, Samoa, Hawaii, Society Islands, Bolabola Island, Tuamotu Archipelago and Marquesas Islands.

Specimens examined: 2♂♂ 1♀, 16. xi. 1937, Toloas-Erin, Truk, Caroline Islands, Esaki leg.; 1♂, 21. i. 1938, Toloas, Truk, Esaki leg.; 2♂♂, 10. vii. 1939, Toloas, Truk, Esaki leg.; 3♂♂ 2♀♀, 3. xii. 1937, Lelo, Kusaie, Caroline Islands, Esaki leg.; 4♂♂, 8. xii. 1937, Mwot—Utwe, Kusaie, Esaki leg.; 1♀, 18. vii. 1939, Ronkiti—One, Ponape, Caroline Islands, Esaki leg.; 1♀, 24. xi. 1937, Wotje-Atoll, Marshall Islands, Esaki leg.; 3♂♂ 2♀♀, 28. xi. 1937, Jabor, Jaluit-Atoll, Marshall Islands, Esaki leg.

Pison korrorense Yasumatsu, 1937

Krombein, 1949, Proc. Hawn. Ent. Soc., 13 : 409; Krombein, 1950, Proc. Hawn. Ent. Soc., 14 : 139, figs. 29, 31, 33. *P. korrorensis* Yasumatsu, 1937, Mushi, 9 : 133 (Palau Islands); Yasumatsu, 1939, Festschr. 60. Geburtst. E. Strand, 5 : 83.

Habitat in Micronesia: Palau Islands—Korror.

Distribution: Palau Islands.

Specimens examined: 1♂ 1♀, 21. ii. 1936, Korror, Korror, Palau Islands, Esaki leg.; 1♀, 20. ii. 1938, Kajangel, Korror, Esaki leg.; 1♂ 5♀♀, 25. viii. 1939, Korror, Korror, Esaki leg.

Pison mariannense sp. nov.

♂. Almost entirely black. Mandibles at the apex castaneous. Tibial calcaria black. Wings strongly infumated with brown and with violaceous reflections, stigma and veins fuscous.

Pubescence silvery, suberect on front, vertex and thorax,

decumbent on clypeus, genae and legs. The apical bands on first to fourth abdominal tergites narrower than those of *P. oakleyi*.

Front shagreened, dull, with sparsely scattered punctures, other portions of the body shining. Punctures on clypeus minute and dense. Punctures on ocellar triangle fine, on vertex fine becoming larger and coarser posteriorly. Impunctate clypeal lobe triangular in out line and higher than that of *oakleyi*. Frontal groove present, but weak, running from anterior ocellus half the distance towards antennal insertions and termination in a short carina. Punctures on the disk of mesonotum very sparsely scattered and slightly larger than those on the posterior portion of vertex. Punctures on mesopleuron comparatively large and coarse, the interspaces about as wide as the width of a puncture. Posterior margin of mesonotum with some short longitudinal wrinkles. Dorsum of propodeum with a distinct median carina except its extremities, some oblique wrinkles or striolae are starting from the base. Posterior surface of propodeum with the usual median impression and about eight or nine strong transverse rugae, not punctured. Lateral surface of propodeum only separated from dorsal surface by a carina and with punctures which are slightly smaller but denser than those on mesopleuron. Punctures on abdomen very minute, delicate and very much coarsely scattered, especially so on the second sternite. Tergites one to five slightly constricted at apices.

Postocellar line three-times the length of oculo-ocellar line. The diameter of posterior ocelli four-times the length of oculo-ocellar line. The diameter of posterior ocelli very slightly longer than or almost as long as postocellar line. Minimum width of front on vertex less than twice the length of the first flagellar segment.

Radial cell very short. Outer cellular line* divided into two straight lines. Petiole of second cubital cell subequal to height of the cell. Third cubital cell: lower side about three-times the length of the upper one. First recurrent nervure interstitial with the first transverse cubital nervure, second recurrent one also interstitial with the second transverse cubital one. Nervulus of fore wings distinctly antefurcal and oblique.

* A line connecting the apex of the radial cell, the third cubital cell and further the outer margin of the second discoidal cell.

Measurements. Length of body: ca. 7 mm. Length of fore wing: ca. 5.5 mm.

♀. Similar to ♂. Clypeal lobe not triangular in out line, but somewhat rounded. Frontal carina longer. Postocellar line twice

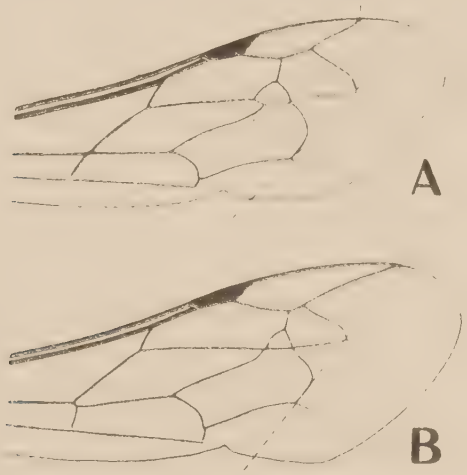

Fig. 2. Fore wings of *Pison mariannense* sp. nov. (A) and the other *Pison*-species (B).

as long as oculo-ocellar line. The diameter of posterior ocelli about twice the length of oculo-ocellar line. The diameter of posterior ocelli about 1.5-times the length of postocellar line. Minimum width of front on vertex about twice the length of the first flagellar segment.

Measurements. Length of body: ca. 8 mm. Length of fore wing: ca. 6 mm.

Habitat in Micronesia: Marianna Islands—Saipan, Rota.

Holotype: 1♂, 5. xi. 1937, Tetêto-Tatâcho-Soñgsoñg, Rota, Marianna Islands, Esaki leg.

Allotype: 1♀, 12. v. 1940, Fanagam, Saipan, Marianna Islands, Yasumatsu et Yoshimura leg.

Paratopotype: 1♂. *Paratype*: 1♀, 3. v. 1940, Matansha—Calabera, Saipan, Yasumatsu et Yoshimura leg.

This new species is quite distinct from all the known species of the genus *Pison* in having a characteristic venation of the fore wings.

Pison nigellum Krombein, 1949

Krombein, 1949, Proc. Hawn. Ent. Soc., 13 : 401 (Ponape); Krombein, 1950, Proc. Hawn. Ent. Soc., 14 : 139, figs. 26, 34, 36 (Ponape).

Habitat in Micronesia: Caroline Islands—Ponape.

Distribution: Caroline Islands.

Specimens examined: 6 ♀♀, 19. xi. 1937, Kolonia—Nat, Ponape, Caroline Islands, Esaki leg.; 9 ♀♀ 1 ♂, 11. i. 1938, Matalanim, Ponape, Esaki leg.; 1 ♀, 14. i. 1938, Nipit—Ronkiti, Ponape, Esaki leg.; 3 ♀♀, 15. i. 1938, Ronkiti—Paliker, Ponape, Esaki leg.; 1 ♀, 16. vii. 1939, Kolonia—Paliker, Ponape, Esaki leg.; 3 ♀♀, 17. vii. 1939, Paliker—Ronkiti, Ponape, Esaki leg.; 3 ♀♀, 18. vii. 1939, Ronkiti—One, Ponape, Esaki leg.; 1 ♀, 22. vii. 1939, Reitao—Oua—U, Ponape, Esaki leg.

Pison oakleyi Krombein, 1949

Krombein, 1949, Proc. Hawn. Ent. Soc., 13 : 406 (Guam, Rota); Krombein, 1950, Proc. Hawn. Ent. Soc., 14 : 139, figs. 27, 30, 32. *P.* sp. Fullaway, Proc. Hawn. Ent. Soc., 2 : 283 (Guam); Swezey, 1942, B. P. Bishop Mus. Bull., 172 : 185 (Guam).

Habitat in Micronesia: Marianna Islands—Pagan, Saipan, Rota, Guam.

Distribution: Marianna Islands.

Specimens examined: 3 ♂♂ 1 ♀, 24. iv. 1940, Soñgsoñg—Regusa, Pagan, Marianna Islands, Yasumatsu et Yoshimura leg.; 3 ♀♀, 8. v. 1940, Matansha-Calabera, Saipan, Marianna Islands, Yasumatsu et Yoshimura leg.; 1 ♀, 12. v. 1940, Fanagam, Saipan, Yasumatsu et Yoshimura leg.; 2 ♂♂, 5. xi. 1937, Tetêto-Tatâcho-Soñgsoñg, Rota, Marianna Islands, Esaki leg.; 1 ♂ 1 ♀, 6. xi. 1937, Soñgsoñg—Taipingot, Rota, Esaki leg.; 1 ♀ (paratype), 17. v. 1936, Taragüe, Guam, Marianna Islands, O. H. Swezey leg.; 1 ♂ (paratype), 19. xii. 1941, Talofofo, Guam, Swezey leg.

Pison ponape Krombein, 1949

Krombein, 1949, Proc. Hawn. Ent. Soc., 13 : 405 (Ponape).

The male (hitherto unknown) differs from the female as follows:

♀	♂
Clypeal lobe rounded.	Clypeal lobe comparatively long and pentagonal in out line.
Postocellar line twice as long as oculo-ocellar line.	Postocellar line as long as oculo-ocellar line.

The diameter of posterior ocelli about three-times the length of oculo-ocellar line.	The diameter of posterior ocelli distinctly longer than oculo-ocellar line.
The diameter of posterior ocelli about 1.5-times the length of postocellar line.	The diameter of posterior ocelli very slightly longer than or almost as long as postocellar line.
Minimum width of front on vertex twice the length of the first flagellar segment.	Minimum width of front on vertex about 1.6-times the length of the first flagellar segment.

Allotype: 1♂, 12. i. 1938, Matalanim—Nipit, Ponape, Caroline Islands, Esaki leg.

Paratypes: 2♂♂, 8. xii. 1937, Mwot—Utwe, Kusaie, Caroline Islands, Esaki leg.

Habitat in Micronesia: Caroline Islands—Ponape, Kusaie.

Distribution: Caroline Islands.

Other specimens examined: 1♀, 15. xii. 1930, Kolonia, Ponape, Caroline Islands, S. Uchiyama leg.; 1♀, 7. vii. 1931, Kolonia, Ponape, Uchiyama leg.; 2♀♀, 19. xi. 1937, Kolonia—Nat, Ponape, Esaki leg.; 1♀, 29. xii. 1937, Kolonia—Paliker, Ponape, Esaki leg.; 1♀, 11. i. 1938, Matalanim, Ponape, Esaki leg.; 1♀, 14. i. 1938, Nipit—Ronkiti, Ponape, Esaki leg.; 2♀♀, 29. i. 1938, Kolonia, Ponape, Esaki leg.; 1♀, 16. vii. 1939, Kolonia—Paliker, Ponape, Esaki leg.; 2♀♀, 18. vii. 1939, Ronkiti—One, Ponape, Esaki leg.; 1♀, 22. vii. 1939, Reitao—Oua—U, Ponape, Esaki leg.; 1♀, 13. xii. 1937, Malem, Kusaie, Esaki leg.

Pison punctifrons Shuckard, 1837

Synonyms: *P. suspiciosus* Smith, 1858; *P. fabricator* Smith, 1869; *P. striolatum* Cameron, 1896; *P. lagunae* Ashmead, 1904; *P. javanum* Cameron, 1905.

Yasumatsu, 1937, Mushi, 9 : 134 (Palau Islands); Yasumatsu, 1939, Festschr. 60. Geburtst. E. Strand, 5 : 83; Krombein, 1949, Proc. Hawn. Ent. Soc., 13 : 400 (Marshall Islands, Caroline Islands, Mariana Islands); Krombein, 1950, Proc. Hawn. Ent. Soc., 14 : 139 (Ponape). *P. rugosus* Semper, 1905, Deutsche Ent. Zeitschr. „Iris", 18 : 246 (Caroline Islands). *P.* sp. Fullaway, Proc. Hawn. Ent.

Soc., 2 : 283 (Guam). *P. lagunae* Swezey, 1942, B. P. Bishop Mus. Bull., 172 : 185 (Guam).

Habitat in Micronesia: Marianna Islands—Pagan, Agrigan, Saipan, Tinian, Guam; Palau Islands—Korror, Babeldaob; Caroline Islands—Yap, Kapingamarangi-Atoll, Ponape, Kusaie; Marshall Islands—Yaluit-Atoll.

Distribution: N. W. India, Ceylon, Burma, Malaya, Sumatra, Java, S. and E. China, Philippines, Pescadores, Formosa, Ishigaki Island, Amami-Oshima, Japan, Bonin Islands, Marianna Islands, Palau Islands, Caroline Islands and Marshall Islands.

Specimens examined: 1♂, 23. iv. 1940, Laguna, Pagan, Marianna Islands, Yasumatsu et Yoshimura leg.; 2♂♂, 28. iv. 1940, Regusa—Tarague, Pagan, Yasumatsu et Yoshimura leg.; 1♀, 3. vii. 1939, Garapan, Saipan, Marianna Islands, Esaki leg.; 2♀♀, 7. v. 1940, Donni—Sadog Tasi, Saipan, Yasumatsu et Yoshimura leg.; 1♂, 2. iii. 1938, Korror, Korror, Palau Islands, Esaki leg.; 1♀, 24. iv. 1938, Korror—Arabaketsu, Korror, Esaki leg.; 1♀, 5. v. 1938, Korror—Arabaketsu, Murakami leg.; 1♀, 25. viii. 1939, Korror, Korror, Esaki leg.; 1♀, 1933, Palau Islands, T. Yoshino leg.; 1♀; vi. 1928, Yap, Caroline Islands, A. Kariya leg.; 1♀, 18. xii. 1937, Malem, Kusaie, Caroline Islands, Esaki leg.; 2♂♂, 19. xi. 1937, Kolonia—Nat, Ponape, Caroline Islands, Esaki leg.; 1♀, 28. xii. 1937, Kolonia, Ponape, Esaki leg.; 1♀, 14. vii. 1939, Kolonia—Sankakuyama, Ponape, Esaki leg.; 3♂♂ 1♀, 28. xi. 1937, Jabor, Jaluit-Atoll, Marshall Islands, Esaki leg.

Pison tahitense Saussure, 1867

Synonym: *P. Rechingeri* Kohl, 1908.

Saussure, 1867, Reise d. Novara, Zool., 2, Hym.: 65 (Tahiti); Perkins et Cheesman, 1928, Insects of Samoa, 5, fasc. 1 : 26 (Samoa); Cheesman, 1928, Ann. Mag. Nat. Hist., ser. 10, 1 : 175 (Marquesas, Society Islands); Williams, 1932, B. P. Bishop Mus. Bull., 98 : 152 (Marquesas); Williams, 1947, Occasional Pap., B. P. Bishop Mus., 18 : 331 (Fiji); Krombein, 1949, Proc. Hawn. Ent. Soc., 13 : 405 (Marshall Islands). *P. Rechingeri* Kohl, 1908, Denkschr. Akad. Wiss. Wien, 81 : 309 (Samoa).

Habitat in Micronesia: Marshall Islands—Ailinglapalap-Atoll.

Distribution: Marshall Islands, Fiji, Samoa, Society Islands, Ellice Islands and Marquesas Islands.

Specimens examined: 1♂ 1♀, 16. vii. 1940, Tapatapao, Upolu, Samoa, C. H. Swezey leg.

Pison trukense sp. nov.

This species is closely related to *P. ponape* Krombein.

ponape ♂	*trukense* ♂
Wings clearly hyaline.	Outer half of fore and hind wings distinctly clouded with brownish colour.
Silvery pubescence on the postero-lateral portions of propodeum shorter.	Silvery pubescence on the postero-lateral portions of propodeum longer.
Dorsum of propodeum without a median carina, but the median sulcus deeper on entire length of surface.	Dorsum of propodeum with a very short basal carina, but the median sulcus shallower on entire length of surface.
Punctures on front sparse.	Punctures on front extremely sparse.
First recurrent nervure received at the first cubital cell just before the first transverse cubitai nervure or just before the second cubital cell.	First recurrent nervure interstitial with the first transverse cubital nervure.
Third cubital cell: lower side about three-times the length of the upper one.	Third cubital cell: lower side about twice the length of the upper one.
Nervulus interstitial.	Nervulus slightly postfurcal.

Measurements of *trukense* ♂. Length of body: ca. 9 mm. Length of fore wing: ca. 7 mm.

trukense ♂	*trukense* ♀
Clypeal lobe longer.	Clypeal lobe much broader.
Postocellar line as long as oculo-ocellar line.	Postocellar line twice the length of oculo-ocellar line.
The diameter of posterior ocelli distinctly longer than oculo-ocellar line.	The diameter of posterior ocelli more than twice the length of oculo-ocellar line.
The diameter of posterior ocelli very slightly longer than or almost as long as postocellar line.	The diameter of posterior ocelli nearly twice the length of postocellar line.
Minimum width of front on	Minimum width of front on

vertex about 1.3-times the length of the first flagellar segment.

vertex as long as the first flagellar segment.

Measurements of *trukense* ♀. Length of body: ca. 9 mm. Length of fore wing: ca. 7 mm.

Habitat in Micronesia: Caroline Islands--Truk, Enderby-Atoll.

Holotype: 1♂, 12. iv. 1940, Olei, Tol, Truk, Caroline Islands, Yasumatsu et Yoshimura leg.

Allotype: 1♀, 10. iv. 1940, Sabote—Epin, Pata, Truk, Yasumatsu et Yoshimura leg.

Paratopotypes: 5♂♂ 1♀. *Paratypes*: 2♀♀, 21. i. 1938, Toloas, Truk, Esaki leg.; 2♂♂, 31. vii. 1939, Kutua, Toloas, Esaki leg.; 1♀, 10. vii. 1939, Toloas, Esaki leg.; 1♀, 5. iv. 1940, Sabote—Epin, Pata, Yasumatsu et Yoshimura leg.; 2♂♂, 6. iv. 1940, Oley--Foup, Tol, Yasumatsu et Yoshimura leg.; 1♂, 3. viii. 1939, Poloat, Enderby-Atoll, Caroline Islands, Esaki leg.

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Journal of the Faculty of Agriculture, Kyūshū University, Vol. 10, No. 2
September 30, 1953

CONTROL OF PINE BEETLES BY THE USE OF ORGANIC CHEMICALS

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Introduction

During the past decade we have experienced increasing damage of pine trees by some Coleopterous insects in Japan. Once infested by these beetles, it has been impossible to kill the beetles alone without destroying the host trees at the same time. Although felling, peeling and burning or trapping have been the standard recommended control methods for years, the control has not always been satisfactory, and the damage has been increasing year after year.

The studies reported in this paper were conducted in an area infested by some of those Coleopterous insects in the Kasuya Experiment Forest, of the Kyushu University, in Sasaguri, Province of Chikuzen, during the spring and autumn seasons of 1949, 1950 and 1951. In the Experiment Forest, the important destructive insects of pine trees were *Myleophilus piniperda* Linné, *Cryphalus fulvus* Niijima, *Xyleborus validus* Eichhoff (Ipidae), *Cryptorrhynchidius insidiosus* Roelofs, *Pissodes nitidus* Roelofs, *Pissodes obscurus* Roelofs, *Sipalus hypocrita* Boheman (Curculionidae) and *Monochamus tesserula* White (Cerambycidae), of which the Ipid beetles were of primary importance, while Curculionid and Cerambycid beetles were of secondary importance. Studies conducted in 1949 showed that *Cryphalus fulvus* was outstanding as the dominant pest in this complex, its damage was almost always antecedent to that of other pests, and consequently the possible complete elimination of this Ipid may have had the advantage of preventing pine trees from the infestation of other beetles.

As is well known, most experimental works on pine beetles control made in Japan have been confined to the direct destruction of insects either by the methods mentioned above or by chemicals. In our studies attempts have been made to make a preliminary test in the evaluation of some chemical repellents for *Cryphalus fulvus*, and the results with some organic chemicals have been encouraging.

Acknowledgments

The present experimentation was undertaken as a cooperative project of the research programme of the Ministry of Education, to which we express our gratitude for financial help. Our deepest thanks are due to Professor T. Esaki, under whose direction the present investigation was carried out and to Professors H. Watanabe, Y. Inoue, S. Ôno and M. Harada for providing facilities for field work at the Kasuya Experiment Forest. Our hearty thanks are also due to Dr. F. P. Keen, Dr. C. H. Hoffmann and Mr. R. Roberts for the gift of many valuable reprints. Acknowledgment is made to Mr. M. Kakei of the Mikasa Chemical Industrial Company, for preparing desired chemicals. Further we are particularly indebted to Messrs. T. Shirôzu, Y. Hirashima, R. Matsuda, A. Nagatomi and Miss C. Ôkuma for assistance in all phases of the experimentation.

Chemicals Used and Experimental Procedure

The chemicals used in this experimentation were supplied from the Mikasa Chemical Industrial Company and included in the following list.

Formula 1. BHC water suspension (BHC 34 % (γ 10 %), bentonite 61 %, adhesive agent 5 %)—γ : 0.05, 0.02, 0.01, 0.008, 0.005, 0.003, 0.001 %.

Formula 2. DDT water suspension (DDT 20 %, bentonite 75 %, adhesive agent 5 %)—DDT : 0.04, 0.02, 0.016, 0.010, 0.0006, 0.002 %.

Formula 3. DDT water emulsion (DDT 20 %, turkey red oil 22 %, cresol 2 %, methyl acetate 1 %, naphtha 55 %)—DDT : 0.1, 0.04, 0.02, 0.016, 0.010, 0.006, 0.002 %.)

Formula 4. BHC water emulsion (BHC 34%, (γ 10%), tween 2%, naphtha 64%)—γ: 0.01, 0.005%.

Formula 5. DDT water emulsion (DDT 20%, turkey red oil 20%, methyl acetate 2%, cresol 2%, naphtha 56%)—DDT: 0.02, 0.01%.

Formula 6. DDT water emulsion (DDT 20%, turkey red oil 20%, methyl acetate 2%, cresol 2%, naphtha 54%, orthodichlorobenzene 2%)—DDT: 0.02, 0.01%.

Formula 7. BHC powder. γ: 0.5%.

In connection with the chemical repellency experiments, small scale tests have been made during 1950 on the phytotoxic effects of these chemicals on the young shoots of pine trees. However, it must be noted that during these experiments no incidents of injury have ever been detected on the foliage from the use of these chemicals.

A row of pine logs used in the field experiments on chemical treatments repellent to the Coleopterous insects.

Uninfested pine tree logs, about three meters in length, suitable for bark beetle attack were used for both checks and treatments, and the bark area was recorded for each log. Sufficient spray or dusting was applied to wet or cover thoroughly the entire bark surface of the material under treatment. Immediately after the treatment both the check and treated logs

were lined out, arranged singly in part shade, and exposed for varying periods of time in the area where bark beetles were known to occur in sufficient numbers. Periodic examinations of these logs were made throughout spring, summer and autumn, and only actual entrance holes of Ipid galleries and oviposition holes of Curculionidae and Cerambycidae were recorded. Thus the difference between the number of entrance holes or oviposition holes in the check logs and in the treated logs gave the measure of repellent effect of the chemicals, and observations on this area were continued to determine how long the difference would continue between these two kinds of logs.

Results

There are four conclusions which are evident from the experimental results secured in 1950. 1. For preventive treatment such chemicals are convenient insecticides, as used in the present experimentation in that they possess the marked advantages of very high repellency effect on *Cryphalus fulvus*. 2. BHC water suspension has a slight repellency effect upon *Myelophilus piniperda* and *Pissodes nitidus*. 3. DDT water suspension possesses a slight repellency effect on *Cryptorrhynchidius insidiosus*. 4. Such chemical treatments are entirely ineffective against *Sipalus hypocrita* and *Monochamus tesserula*. As is mentioned in the introduction, *Cryphalus fulvus* is exclusively abundant in number, and its damage is almost always antecedent to those of other beetles. For such reasons together with the first conclusion mentioned above, our repellency experiments have been concentrated against this bark beetle. The results of the experiments and accumulated rainfall in the experimental periods are given in Tables 1 to 7.

Table 1. Experiment 1—Repellent effect of chemicals applied to pine logs on April 21st, 23rd*, 29th† and May 13th** respectively. (Period of experiment: 21. iv.—1. vii. 1950).

Test No.	Chemical formula	Entrance holes per square foot of bark area										
		After 3 days	5	9	15	19	23	34	40	47	56	72
3	Check	—	—	—	—	—	—	—	2.0	3.0	5.5	6.4
5	„	0.2	0.3	0.7	0.7	0.7	0.7	0.7	1.3	3.9	6.1	7.3
6	„	—	—	—	—	—	—	0.1	1.3	6.6	8.6	10.2

7	„	—	—	—	—	—	—	0.1	0.3	2.4	4.6	6.5
8	„	—	0.1	0.2	0.2	0.9	1.3	1.8	4.0	5.7	10.1	10.1
9	„	—	—	0.4	0.4	0.7	0.7	3.9	6.0	7.6	8.8	9.1
10	„	—	—	0.1	0.1	0.1	0.1	7.1	10.6	13.0	14.7	16.0
11	„	—	—	—	—	—	—	7.1	9.4	17.1	19.2	21.4
12	„	—	—	0.1	0.1	0.3	0.7	1.7	4.5	16.3	20.3	20.7
13	„	0.1	0.1	0.1	0.1	0.4	0.4	5.8	8.1	11.6	13.5	13.5
14	„	—	—	0.2	0.2	3.9	3.9	10.0	15.8	20.0	24.3	24.3
16	B1	—*	—	—	—	—	—**	—	—	—	—	0.9
17	B2	—*	—	—†	—	—	—	0.1	0.1	0.1	0.2	1.1
18	B3	—*	—	—†	—	—	—**	—	—	—	—	—

B1 : BHC water suspension (γ 0.02 %). B2 : DDT water suspension (DDT 0.04 %). B3 : DDT water emulsion (DDT 0.04 %). Accumulated rainfall : more than 470 mm. Heavy rainfall : 26. iv., 12. v., 20. v., 16. vi., 18. vi., 19. vi., 20. vi., 21. vi., 29. vi.

Table 2. Experiment 2—Repellent effect of chemicals applied to pine logs on April 25th and May 5th* respectively.
(Period of experiment: 25. iv.—15. vi. 1950).

Test No.	Chemical formula	Entrance holes per square foot of bark area						
		11	15	19	31	36	43	52
19	Check	—	—	—	—	0.1	0.5	2.5
20	„	—	—	—	11.6	11.6	11.6	11.6
21	„	—	0.1	0.1	0.1	4.1	5.1	5.4
22	„	—	—	—	4.9	8.8	11.8	15.0
23	„	—	0.9	0.9	5.0	12.3	17.1	19.8
24	„	—	0.3	0.3	0.3	4.6	10.0	11.6
25	„	—	—	—	2.4	9.1	13.0	13.7
26	B5	—*	—	—	—	—	—	—
27	B6	—*	—	—	—	—	—	—
28	B7	—*	—	—	—	—	—	—

B5 : BHC water suspension (γ 0.02 %). B6 : DDT water suspension (DDT 0.04 %). B7 : DDT water emulsion (DDT 0.04 %). Accumulated rainfall: more than 200 mm. Heavy rainfall : 26. iv., 12. v., 20. v.

Table 3. Experiment 3—Repellent effect of chemicals applied to pine logs on May 9th. (Period of experiment: 9. v.—25. vii. 1950).

Test No.	Chemical formula	Entrance holes per square foot of bark area					
		16	22	29	38	54	79
29	Check	0.2	0.2	0.3	0.5	0.5	1.8
30	„	0.3	1.1	1.3	1.3	2.8	6.6
31	„	0.8	4.8	14.2	16.2	19.9	20.2
32	„	0.4	2.3	3.9	5.0	10.0	11.3
33	„	1.4	20.9	31.0	34.0	39.4	47.0
34	„	1.9	13.1	19.3	19.6	21.6	26.2
35	„	12.6	49.7	62.7	66.8	72.7	75.9
36	B9	—	—	—	—	—	—
37	B10	—	—	—	—	—	0.1
38	B11	—	—	—	—	—	—
39	B12	0.1	0.1	0.3	0.3	0.3	7.2
40	B13	—	0.5	0.7	0.7	0.9	3.3
41	B14	—	0.1	0.1	0.5	1.8	7.3

B9 : BHC water suspension (γ 0.05%). B10 : BHC water suspension (γ 0.02%). B11: BHC water suspension (γ 0.01%). B12 : DDT water emulsion (DDT 0.1%). B13 : DDT water emulsion (DDT 0.04%). B14 : DDT water emulsion (DDT 0.02%). Accumulated rainfall : more than 600 mm. Heavy rainfall : 12. v., 20 v., 16. vi., 18—21. vi., 29. vi., 2. vii., 5. vii.

Table 4. Experiment 4—Repellent effect of chemicals applied to pine logs on June 25th.
(Period of experiment : 25. vi.—23. viii. 1950).

Test No.	Chemical formula	Entrance holes per square foot of bark area			
		7	34	49	60
42	Check	—	3.4	5.4	5.4
43	„	—	30.6	37.5	38.5
44	„	—	57.6	70.0	71.9
45	„	—	75.1	88.3	90.6
46	„	—	130.9	158.1	160.5
47	B25	—	1.2	7.1	8.0
48	B26	0.1	0.8	3.8	5.2
49	B27	—	0.5	8.1	8.8

50	B28	—	7.2	16.1	17.3
51	B29	0.1	7.0	16.2	19.3
52	B30	1.1	13.8	26.4	32.1
53	B31	—	0.5	7.3	9.4
54	B32	—	1.3	7.0	8.1
55	B33	—	0.8	4.7	6.0

B25 : BHC water suspension (γ 0.008 %). B26 : BHC water suspension (γ 0.005 %). B27 : BHC water suspension (γ 0.003 %). B28 : DDT water suspension (DDT 0.016 %). B29 : DDT water suspension (DDT 0.010 %). B30 : DDT water suspension (DDT 0.006 %). B31 : DDT water emulsion (DDT 0.016 %). B32 : DDT water emulsion (DDT 0.010 %). B33 : DDT water emulsion (DDT 0.006 %). Accumulated rainfall : more than 500 mm. Heavy rainfall : 29. vi., 2. vii., 5. vii., 16. viii., 17. viii.

Table 5. Experiment 5—Repellent effect of chemicals applied to pine logs on August 7th.
(Period of experiment : 22. vii.—5. ix. 1950).

Test No.	Chemical formula	Entrance holes per square foot of bark area				
		4	8	21	31	46
56	Check	—	15.1	15.6	19.4	23.5
57	„	—	6.7	17.5	22.1	25.3
58	„	0.8	29.0	45.7	55.2	60.0
59	B34	—	—	0.1	0.3	1.0
60	B35	—	0.2	0.2	0.4	1.0
61	B36	—	6.1	8.3	10.6	12.1
62	B37	—	2.4	4.8	11.9	16.1
63	B38	—	1.5	2.3	3.8	5.8
64	B39	—	2.7	4.0	6.7	9.1
65	B40	—	—	—	—	—
66	B41	—	0.1	0.4	0.7	1.1
67	B42	—	0.3	0.5	2.9	6.1

B34 : BHC water suspension (γ 0.008 %). B35 : BHC water suspension (γ 0.005 %). B36 : BHC water suspension (γ 0.003 %). B37 : DDT water suspension (DDT 0.016 %). B38 : DDT water suspension (DDT 0.010 %). B39 : DDT water suspension (DDT 0.006 %). B40 : DDT water emulsion (DDT 0.016 %). B41 : DDT water emulsion (DDT 0.010 %). B42 : DDT water emulsion (DDT 0.006 %). Accumulated rainfall : more than 350 mm. Heavy rainfall : 16. vii., 28. viii., 30. vii., 31. viii.

Table 6. Experiment 6—Repellent effect of chemicals applied to pine logs on August 7th.
(Period of experiment: 7. viii.—28. ix. 1950).

Test No.	Chemical formula	Entrance holes per square foot of bark area		
		15	30	53
68	Check	1.1	1.6	1.8
69	„	—	0.1	0.1
70	„	2.2	2.5	3.2
71	B43	—	0.1	3.0
72	B44	0.1	0.2	0.8
73	B45	0.4	1.0	2.6
74	B46	3.4	3.9	9.7
75	B47	1.6	2.6	8.2
76	B48	4.3	4.8	18.8
77	B49	—	—	—
78	B50	0.1	0.1	0.2
79	B51	0.5	1.0	6.9

B43 : BHC water suspension (γ 0.008 %). B44 : BHC water suspension (γ 0.005 %). B45 : BHC water suspension (γ 0.003 %). B46 : DDT water suspension (DDT 0.016 %). B47 : DDT water suspension (DDT 0.010 %). B48 : DDT water suspension (DDT 0.006 %). B49 : DDT water emulsion (DDT 0.016 %). B50 : DDT water emulsion (DDT 0.010 %). B51 : DDT water emulsion (DDT 0.006 %). Accumulated rainfall : more than 150 mm. Heavy rainfall : 16. viii., 17. viii., 28. viii., 30. viii., 31. viii., 19. ix., 22. ix.

Table 7. Experiment 7—Repellent effect of chemicals applied to pine logs on September 5th.
(Period of experiment: 5. ix.—19. ix. 1951).

Test No.	Chemical formula	Entrance holes per square foot of bark area		Test No.	Chemical formula	Entrance holes per square foot of bark area	
		7	14			7	14
80	Check	18.8	36.4	87	B70	0.1	0.7
81	„	15.0	39.4	88	B71	0.4	1.3
82	„	10.9	21.9	89	B72	0.1	0.7
83	B66	0.7	1.7	90	B73	0.4	1.7
84	B67	0.8	2.1	91	B74	0.4	2.9
85	B68	0.1	4.3	92	B75	1.3	5.0
86	B69	0.3	1.4	93	B76	0.1	3.3

B66 : DDT water suspension (DDT 0.02 %). B67 : DDT water suspension (DDT 0.01 %). B68 : DDT water emulsion (DDT 0.02 %). B69 : DDT water

emulsion (DDT 0.01%). B70 : DDT water emulsion No. 6 (DDT 0.02%). B71 : DDT water emulsion No. 6 (DDT 0.01%). B72 : DDT water emulsion No. 5 (DDT 0.02%). B73 : DDT water emulsion No. 5 (DDT 0.01%). B74 : BHC water emulsion (γ 0.01%). B75 : BHC water emulsion (γ 0.01%). B75 : BHC water emulsion (γ 0.005%). B76 : BHC dust (γ 0.5%). Accumulated rainfall : more than 250 mm. Heavy rainfall : 13. ix., 14. ix., 16. ix.

The following conclusions may be derived from an examination of the data presented in tables given above. In general all the chemicals used in the experiments have repellency effect against *Cryphalus fulvus* to a more or less extent. DDT is the most effective against this bark beetle when it is used in the form of a water emulsion. BHC and DDT are also effective repellents, when they are used in the form of a water suspension. BHC dust (γ 0.5%) is promising under condition of a small amount of rainfall. BHC water suspension (formula 1) (γ : 0.003, 0.001%), DDT water suspension (formula 2) (DDT : 0.006, 0.002%), and DDT water emulsion (formula 3) (DDT : 0.006, 0.002%) are ineffective as repellents. BHC water suspension (formula 1) (γ : 0.05, 0.02, 0.01, 0.008, 0.005%), DDT water suspension (formula 2) (DDT : 0.04, 0.02, 0.016, 0.010%), DDT water emulsion (formuia 3) (DDT : 0.1, 0.04, 0.02, 0.016, 0.010%), BHC water emulsion (formula 4) (γ : 0.01, 0.005%), DDT water emulsion (formula 5) (DDT : 0.02, 0.01%), and DDT water emulsion (formula 6) (DDT : 0.02, 0.01%) are suitable for the preventive treatment against infestation by this bark beetle. There are found no definite difference in the repellency effect of BHC water suspension of γ−contents between 0.05 and 0.01%. There are also found no marked difference in repellency of DDT water suspension and emulsion of DDT contents between 0.1 and 0.02%. The residual effect of these chemicals lasts at least two weeks. Under favourable conditions the repellency effect lasts more than two months. It would seem that there is wide variation in repellency of a given formula under similar conditions and there are considerable logs which are virtually almost immune to infestation by *Cryphalus fulvus*. But it is at present impossible to determine whether these phenomena are ascribed to some physiological or racial properties of the tree itself. Under conditions of heavy rainfall, a higher dosage of DDT and BHC or two or three successive

applications at an interval of a week may be necessary as the movement of these chemicals would be more rapid.

Considerations

Very little scientific information has been published on the satisfactory protection of living trees from infestation of bark beetles by the use of organic chemicals. The paper giving the most interesting information was one by G. H. Plumb (1950). The primary objective of his study was to determine whether or not large elm trees in an area of high infestation rate could be protected by spray directed against the bark beetle vectors of Dutch elm disease. He used a DDT emulsion throughout his experiment in Connecticut, and DDT has shown to be highly effective against *Scolytus multistriatus*. Industrial grade of Xylene was the solvent and an emulsifying agent was used. An emulsion of 12.5 per cent was used as a dormant spray, and one of 6.25 per cent as a summer spray when the trees were in leaf. Periodic examinations of the trees were made throughout each summer for evidence of disease. The final inspection in 1948, made in early September, indicates that 3 trees, or 2.9 per cent of the sprayed trees; and 21, or 21.0 per cent of the check trees, were infested. At the end of August, 1949, 9 trees, or 8.9 per cent of the trees sprayed; and 39, or 38.6 per cent of the check trees, were diseased.

When we planned to conduct our experimentation in 1949, we were unaware of the study of Plumb. In 1951 we have had the good fortune to find Plumb's paper and thereby recognized that the objective of our study was quite similar to that of Plumb. It is now clear from our experimentation that DDT and BHC are highly suitable repellents for the prevention treatment of pine trees against infestation by *Cryphalus fulvus*. Namely, an application of the lowest dosage, 0.005 per cent of ; BHC or 0.01 per cent DDT was satisfactory in preventing the bark beetle attack.

According to the unpublished data secured by Kaku, one of the authors, the adult *Cryphalus fulvus* occurs three times a year in our province, viz., April to June (maximum in May), June to August (maximum in July) and September to November (maximum at the end of October). The adults are most abundant in July

and quite few in October in comparison with those in May. Therefore, more attention would need to be given to applying DDT or BHC at a time coinciding with the emergence of the adult bark beetles. Ideally these chemicals should be applied immediately before the emergence of the adult bark beetles. If we can eliminate this bark beetle in its first and second generations, the emergence of the third brood would become negligible.

Having determined that prevention of pine trees from *Cryphalus fulvus* by the use of repellents is possible under experimental conditions, it becomes desirable to test our results in the forest and a means of applying such chemicals which involve the use of conventional equipment and which could be employed by some sprayers or dusters at a minimum cost. In conjunction with the practical application of these repellents in the forest, it must be borne in mind that these chemicals have certain characteristics that may limit their use as a control for the bark beetles. One of those characteristics may be seen in their extensive survey made by Hoffmann and al. (1948, 1949), and their general conclusions are cited in the following lines. "A single airplane application of DDT to a forest at the rate of 1 pound per acre—enough to control many forest pests—does not seriously damage the general arthropod fauna, although a few species would probably be exterminated by a thorough application at this rate. DDT used at moderately light dosage will tend to restrict the range and abundance of many harmless and beneficial species, and its widespread use on forest threatens extermination to some of the more susceptible and sedentary species. However, even with a dosage of 5 pounds of DDT per acre, the effect on the arthropod fauna as a whole is far from being calamitous." Thus it seems possible that the application of these repellents within the limit derived from the survey of Hoffmann and al. should prevent the pine trees from attack by bark beetles in question without damaging the general Arthropod fauna and result the shortage of food for the bark beetle populations in a given area.

In their studies on the protection of elm wood from attack by bark beetles, R. R. Whitten (1942) and D. P. Connola et al. (1947) experienced the wide variation in repellency of a given formula under similar experimental conditions. This phenomenon was also observed in our experiments. Further careful experi-

ments by applying the statistical method may throw some light upon the analysis of this phenomenon, and it would be expected that at least the specific behaviour of the bark beetles and the nature of the pine tree itself would be responsible for the analysis.

Summary

Field studies on the control of pine beetles were conducted with some organic chemicals in the Kasuya Experiment Forest, Province of Chikuzen.

Cryphalus fulvus is by far the most important and harmful bark beetle among others. The control of this bark beetle results the prevention of attack of all other beetles on the living pine trees.

Both DDT and BHC have an excellent repellency effect on *Cryphalus fulvus* at the lowest dosage of 0.01 per cent DDT or 0.005 per cent BHC spray without giving any phytotoxic effects even on the young shoots of pine trees.

Under favourable conditions the repellency effect lasts more than two months.

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Journal of the Faculty of Agriculture, Kyūshū University, Vol. 10, No. 2
September 30, 1951

A NEW EULOPHID PARASITE OF *ADRIS TYRANNUS* GUENEE FROM JAPAN

(Hym., Eulophidae)

Keizô Yasumatsu

The citrus orchards of Japan have long been sustaining attacks by Noctuid moths which have caused comparatively serious damage by piercing the rind of the citrus fruits with their probosces.

While studying the biology of *Adris tyrannus* Guénée, one of the fruit-piercing Noctuidae, in Miyazaki Prefecture, Kyushu, Mr. S. Nasu found an Eulophid parasitic on the larvae of this moth. Professor S. Nakajima, of the Miyazaki University, handed the specimens of this parasite over to me for identification.

After carefully examining the material submitted to me, I came to the conclusion that the specimens presented a new species. Meanwhile, I sent some specimens of this parasite to Dr. B. D. Burks, Hymenopterist of the U. S. National Museum (to whom I was referred by Mr. Karl V. Krombein) and to Dr. Ch. Ferrière, Hymenopterist of the Geneva Museum, to confirm my conclusion. I further borrowed Girault's type specimens of *Euplectrus*-species from Dr. G. Mack, Director of the Queensland Museum.

I am deeply indebted to Dr. B. D. Burks for his kindness in comparing specimens under his charge and for his generous criticism to my manuscript. I am also indebted very much to Dr. Ch. Ferrière for his valuable advice and for the donation of valuable reprints, and to Dr. G. Mack for the loan of precious type specimens of *Euplectrus* under his charge. Further I am indebted for constant guidance and encouragement to Professor T. Esaki; for many favours to Mr. Karl V. Krombein; for literature to Mr. K. Maruyama; and for the donation of specimens to Professor S. Nakajima and Mr. S. Nasu, respectively.

A Tentative Key to the Species of the Genus *Euplectrus* Swederus from Japan and Formosa

1. Head and thorax partly yellow ..... *nigromaculatus* Ashmead
— Head and thorax black 2
2. Hind coxae black ..... *japonicus* Ashmead
— All coxae pale 3
3. Scutellum at base deeply reticulated, apically longitudinally rugose ..... *kuwanae* Crawford
— Scutellum finely, indistinctly reticulated 4
4. Clypeal area black 5
— Clypeal area pale 6
5. First funicle segment not distinctly longer than pedicel ..... *koebelei* Crawford
— First funicle segment much longer than pedicel ..... *taiwanus* Sonan
6. Mesoscutum with a complete, longitudinal, median carina ..... *noctuidiphagus* n. sp.
— Mesoscutum without such a median carina ..... *fukaii* Crawford

Euplectrus noctuidiphagus n. sp.

♂♀. Head and thorax black, clypeal area whitish; antennae orange-yellow, brownish apically; antennal scape of male pale, pedicel and funicle darker or brownish apically; legs entirely orange-yellow, legs of male much paler; abdomen orange-yellow above and below, black only on the sides and the apical half. Vertex and thorax with some scattered, long, whitish ciliae.

♀. Head very transverse, almost smooth; vertex with a fine but distinct carina extending completely across its posterior margin; the lateral ocelli closer to the front ocellus than to the eye margins; eyes large and oval, not ciliate. Antennae with the scape narrow, its apex reaching the level of the front ocellus; scape, pedicel and the first funicle segment of the same width; scape long, about six times as long as wide, pedicel about twice as long as wide at the apex; relative lengths of scape, pedicel and the first funicle segment—65 : 18 : 40, the following segments shorter. Pronotum short, with a distinct, transverse carina comparatively far from the posterior margin. Mesonotum irregularly, transversely striate in the middle; mesoscutum with a complete, longitudinal, median carina. Axillae almost smooth. Scutellum

finely reticulate. Propodeum smooth, shining, with a distinct, complete, median, longitudinal carina and the spiracles distinctly oblong oval. The longest hind tibial spur almost as long as the first two tarsal segments taken together. Wings large; marginal vein slightly longer than the submarginal vein, the costal cell slightly broadened, relative lengths of the stigmal and the postmarginal veins—5 : 9, marginal vein about four times as long as the stigmal vein. Abdomen broadened behind, triangular, shorter than the thorax; abdominal petiole short, subquadrate or slightly wider than long, shagreened and with a complete, longitudinal, median carina.

♂. Similar; relative lengths of the scape, pedicel and the first funicle segment—50 : 15 : 40, scape especially broadened in the middle, less than three times as wide as long, scape much wider than pedicel, pedicel slightly but distinctly longer than wide and very slightly wider than the first funicle segment which is as long as the third one, first funicle segment distinctly longer than the second one which is as long as fourth one. Abdomen much narrower than that of the female, petiole without a longitudinal carina.

Length of body: ♂ about 2.0 mm. ♀ about 3.5 mm.

Holotype: ♂, 13. x. 1951, Miyazaki, Kyushu (Mr. S. Nasu), ex a larva of *Adris tyrannus* Guénée.

Allotype: ♀, 19. x. 1951, the same data as the holotype.

Paratypes: 15♂♂, 13. x. 1951, 13♀♀, 19. x. 1951.

Holo-, allo- and 20 paratypes are preserved in the collection of the Entomological Laboratory, Kyushu University, Fukuoka. Eight paratypes will be distributed to the British Museum (Natural History), U. S. National Museum, American Museum of Natural History, and the Geneva Museum.

Habitat: Japan.

This new species is somewhat allied to *Euplectrus taiwanus* Sonan, 1942, from Formosa, but differs from the latter in the following points:—1. clypeal area whitish, 2. head smooth and shining, without any reticulated, impressed lines, 3. propodeum without a crenulate carina along the basal margin, 4. abdominal petiole without longitudinal striations. Judging from the description, the new species is also related to *Euplectrus kuwanae* Crawford, 1911, from Japan, the types of which are deposited in the

U. S. National Museum. Dr. Burks was so kind as to compare my specimen not only with the type of *kuwanae* but also all the specimens of the other species in his trust and wrote me as follows:—"I have compared it with the type of *E. kuwanae* and find that it is not that species. I checked it against all the species of this genus in our collection, but I am sorry to say I cannot place it. It is nearest to *agaristae* Crawford, from Australia, and is also quite close to the cosmopolitan *plathypenae* Howard, but is not identical with either. It has a complete, longitudinal, median carina on the mesoscutum and there is a fine but clearly discernible carina extending completely across the posterior margin of the vertex. These characters, along with the color, distinguish your specimen from all the species of this genus in our collection" (February 15, 1952).

The genus *Euplectrus* is parasitic on larvae of Lepidoptera. Since no concise world list of the known host Lepidoptera of the genus has been published hitherto, it has been considered appropriate to attempt to summarize the available data regarding the *Euplectrus*-species that have been recorded from the pests of crops.

A List of Host of *Euplectrus*

Species	Hosts	Localities
agaristae Crawford	*Phalaenoides glycinae* Lewin	Australia
bicolor Swederus	*Amathes c-nigrum* Linné	U. S. A.
	Aplecta nebulosa Hufnagel	Europe
	"Crino setara"	Europe
	Epiglaea apicata Grote	U. S. A.
	Etiella zinckenella Treischke	Europe
	Laphygma exigua Hübner	Anatolia
	"Miselia tincta"	Europe
cacoeciae Ferrière	*Archips rosana* Linné	Europe
catocalae Howard	*Alabama argillacea* Hübner	U. S. A.
	Autographa sp.	U. S. A.
	Heliothis armigera Hübner	U. S. A.
	Laphygma frugiperda Smith et Abbot	U. S. A.
	Plathypena scabra Fabricius	U. S. A.
	Prodenia ornithogalli Guénée	U. S. A.

	Trichoplusia ni Hübner	U. S. A.
ceylonensis Howard	*Euproctis fraterna* Moore	Ceylon
	Euproctis flava Bremer	Ceylon
	Nygmia scintillans Walker	Malaya
comstocki Howard	*Alabama argillacea* Hübner	U. S. A.
	Autographa sp.	U. S. A.
	Caradrina sp.	U. S. A.
	Heliothis armigera Hübner	U. S. A.
	Laphygma frugiperda Smith et Abbot	Puerto Rico, Trinidad
	Plathypena scabra Fabricius	U. S. A.
	Trichoplusia ni Hübner	U. S. A.
epiplemae Ferrière	*Epiplema dohertyi* Warren	Africa
euplexiae Rohwer	*Perigea capensis* Guénée	India
	Selepa docilis Butler	India
frontalis Howard	*Amathes c-nigrum* Linné	U. S. A.
fukaii Crawford	*Naranga aenescens* Moore	Japan
furnius Walker	*Protoparce cingulata* Fabricius	Barbados
gopimohai Mani	*Laphygma exigua* Hübner	India
howardi Olliff	*Phragmatiphila truncata* Walker	Australia
junctus Gahan	*Isoparce cupressi* Boisduval	U. S. A.
kuwanae Crawford	*Parnara guttata* Bremer et Grey	Japan
laphygmae Ferrière	*Amsacta moloneyi* Druce	Africa
	Heliothis sp.	Africa
	Laphygma exempta Walker	Africa
	Laphygma exigua Hübner	Africa
	Leucania obsoleta Fabricius	Africa
	Phytometra gamma Linné	Africa
	Plusia orichalcea Fabricius	Africa
leonae Risbec	*Anomis leona* Schaus	Africa
leucostomus Rohwer	*Achaea janata* Linné	India
	Trabala vishnu Lefebvre?	India
liparidis Ferrière	*Lymantria dispar* Linné	Africa
manilae Ashmead	*Anomis* sp.	Philippines
	Cosmophila sp.	Philippines
	Papilio alphenor Cramer	Philippines
maternus Bhatnagar	*Orthreis fullonica* Linné	India
	Orthreis materna Linné	India
mellipes Provancher	*Coleophora laricella* Hübner	Canada
	Feralia jocosa Guénée	Canada

noctuidiphagus Yasumatsu	*Adris tyrannus* Guénée	Japan
nyctemerae Crawford	*Nyctemera laticinctia* Cramer	India
parvulus Ferrière	*Boarmia selenaria imparata* Walker	India
	Plecoptera reflexa Gmelin	India
	Tephria disputaria Gmelin	India
phthorimaeae Ferrière	*Gnorimoschema operculella* Zeller	Cyprus
phytometrae Risbec	*Phytometra* sp.	Africa
plathypenae Howard	*Autographa falcifera simplex* Guénée	U. S. A.
	Cirphis humidicola Guénée	Br. West Indies
	Cirphis unipuncta Haworth	U. S. A., Hawaii
	Diatraea saccharalis Fabricius	U. S. A.
	Heliothis armigera Hübner	U. S. A., Hawaii
	Laphygma exempta Walker	Hawaii
	Laphygma exigua Hübner	U. S. A.
	Laphygma frugiperda Smith et Abbot	U. S. A., Cuba
	Leucania latiuscula Herrich-Schäffer	U. S. A., Mexico
	Leucania multilinea Walker ?	U. S. A.
	Lycophotia margaritosa Haworth	Hawaii
	Peridroma margarita Haworth	U. S. A.
	Plathypena scabra Fabricius	U. S. A.
	Prodenia ornithogalli Guénée	U. S. A.
	Prodenia sunia Guénée	U. S. A.
	Protoparce sexta Johanssen	U. S. A.
singularis Ferrière	*Anaphaeis creona creona* Cramer	Africa
spodopterae Bhatnagar	*Cirphis* sp.	India
	Spodoptera mauritia Boisduval	India
taiwanus Sonan	*Bombotelia jocosatrix* Guénée	Formosa
	Euproctis taiwana Shiraki	Formosa
	Spodoptera mauritia Boisduval	Formosa
utethesiae Mani et Kurian	*Utethesia pulchella* Linné	India

Journal of the Faculty of Agriculture, Kyūshū University, Vol. 10, No. 2
September 30, 1953

EGG-LAYING HABITS OF A SPINACH LEAF-MINER, *PEGOMYIA HYOSCYAMI* PANZER (Diptera)

Keizô Yasumatsu and Mitsuhiro Sasagawa

Introduction

The first published record of the infestation of spinach by *Pegomyia hyoscyami* Panzer in Japan is found in a booklet made by Oda and Takimoto (1939), in which the fly is reported in Fukuoka Prefecture. In view of the widespread existence and abundance of the fly in Northern Kyushu since 1939, there is reason to believe that it increased markedly some time before that year. The senior author assumes that this increase may chiefly be attributed to the cultivation of some European and Chinese races of spinach. Before the cultivation of such spinaches, several weeds of the family Chenopodiaceae were the only preferred host plants to this fly. Thus, a serious menace to the successful cultivation of spinach in almost all the spinach growing areas came to the senior author's attention in 1943, leading to observations on the bionomics of the fly. The present report is a part of the authors' investigations which were made during 1943 to 1947 near and in the campus of Kyushu University, Fukuoka.

This opportunity is taken to acknowledge the kind guidance given by Professor Teiso Esaki of Kyushu University. Acknowledgment is also due to Dr. Satoru Kuwayama, of the Hokkaido Agricultural Experiment Station, for his kindness in literature. Much credit is also due Professor Hajime Yoshii for his kind suggestion and Mr. Kazuo Yasutomi for his helpful assistance in material.

Degree of Infestation or the Number of Eggs Deposited on a Single Host Plant

Experience in previous years and observations in 1943 indicated that 100 per cent loss of spinach occurred even if one egg of *Pegomyia hyoscyami* was deposited on every leaf of spinach plant. In Table 1 are shown some samples of completely damaged spinach plants observed in a spinach area near the campus of Kyushu University in the spring of 1945. Spinach plant No. 1 has 53 leaves with as many eggs as 449. This figure highly exceeds the highest record of Kemner (1925) who observed one sugar beet habouring 304 eggs in Sweden. As seen in Table 1, the number of eggs per leaf ranged from 6.3 to 11.3. Such state of high egg populations would indicate the complete damage of spinach field, which, in turn, becomes a source of infestation in other spinaches growing on the same or a neighbouring farm within the same or another season. Influences by the recent increased damage done by *Pegomyia hyoscyami*, cultivation areas of spinach of foreign races have become narrowed and received considerable degree of financial losses.

Table 1. Number of eggs deposited on five plants of an European race of spinach, *Viroflay*, which were selected at random in the spinach area near the campus of Kyushu University in the spring of 1945.

No. of eggs laid	Spinach No. 1		2		3		4		5	
	A	B	A	B	A	B	A	B	A	B
2	3	6					1	2		
3	4	12	2	6			1	3	1	3
4	9	36	4	16	5	20	6	24		
5	9	45	1	5	1	5	2	10		
6	5	30	2	12	4	24	1	6	1	6
7							1	7		
8	4	32	1	8			1	8	1	8
9	1	3	2	18	1	9				
10	5	50	2	20			1	10		
11	4	44	1	11			1	11		
12	3	36	1	12			1	12		

13	1	13					1	13		
14					1	14				
15	1	15	1	15					1	15
16									1	16
17										
18										
19										
20			1	20						
21			2	42	1	21				
22			1	22	1	22				
23	1	23								
24										
25										
26			1	26						
27										
28										
29	1	29								
30	1	30								
31										
32					1	32				
33										
34					1	34				
35										
36										
37										
38										
39	1	39								
Total	53	449	21	233	16	181	17	106	5	48
No. of eggs per leaf	8.4		11.0		11.3		6.3		9.6	

A : Number of leaves. B : Total number of eggs.

Preoviposition Period and the Number of Eggs Laid

To ascertain the preoviposition period and the total number of eggs laid by one female, five couples of newly emerged individuals were isolated in pairs and each pair were confined into a separate cage which was provided with fresh spinach leaves,

and the date and number of eggs laid by each female were recorded. The flies were fed with honey, and the cages containing flies were placed near the window partly so as to receive sunlight. Thus the temperature of each cage was found to be almost equal to that in the field. The record of these observations are shown in Table 2.

Table 2. Preoviposition period and the number of eggs laid by five mated females of *Pegomyia hyoscyami* (Observation made in 1943).

No. of individuals	1		2		3		4		5	
Date	M	N	M	N	M	N	M	N	M	N
18. v.	Emerged				Emerged					
19. v.							Emerged		Emerged	
20. v.			Emerged							
21. v.										
22. v.										
23. v.										
24. v.										
25. v.	4	11								
26. v.										
27. v.	9	30	11	29						
28. v.	1	3								
29. v.					3	5	2	12		
30. v.					29	72	4	28	7	43
31. v.			1	1			12	33	2	10
1. vi.							21	36		
2. vi.	1	3					2	4	1	4
3. vi.										
4. vi.		*(A)	1	1					2	7
5. vi.				*(B)						
6. vi.								*(D)		
7. vi.						*(C)				
8. vi.										
9. vi.										*(E)
Total	15	47	13	31	32	77	32	113	12	64
Preoviposition period	7 days		7 days		11 days		11 days		12 days	

* Asterisk denotes the date of death. Upon dissection the female still contained some well-developed eggs. (A: 15 eggs, B: 22 eggs, C: 28 eggs, D: 14 eggs, E: 30 eggs, M: number of egg-masses, N: number of eggs).

Generally, the mating took place on the day of emergence, sometimes just after emergence. A series of experiments showed that the virgin females or females fed with sugar solution only do not deposit any eggs. The food of the flies in the field is partly the nectar of flowers. As shown in Table 2, the preoviposition period varied about 7 to 12 days. Of course this period has a close connection to the nutrition of the female and the temperature of the environment. For example, Cory (1916) recorded 4 days in U. S. A., and Hille Ris Lambers (1933) observed 5 days in Holland. The maximum number of eggs laid per female was 113. Zolk (1932) counted 112 eggs which were deposited by a single female in Estonia. According to the experiment made by Bremer and Kaufmann (1931) in Germany, the maximum number of eggs deposited per female was 191 under laboratory conditions.

Number of Eggs in a Single Egg-Mass

Number of eggs in a single egg-mass varies considerably. For example, Vassiliev (1914) in Russia wrote 2 to 12 eggs (average 4.5 eggs) comprising one egg-mass. Rambousek (1925) in Cechoslovakia recorded 1 to 18 eggs in a single egg-mass. Kemner (1925) in Sweden recorded 1 to 12 eggs in an egg-mass. Takiguchi (1948) in Japan reported 1 to 7 eggs comprising one egg-mass. On April, 15, 1945, the authors collected a number of leaves of *Chenopodium centrorubrum* in the field and observed the number of eggs comprising one egg-mass. The results are summarized in Table 3.

Table 3. Number of eggs in a single egg-mass of *Pegomyia hyoscyami*.

No. of eggs	No. of egg-masses	No. of eggs	No. of egg-masses	No. of eggs	No. of egg-masses
1	7	7	25	13	1
2	12	8	12	14	—
3	40	9	8	15	—
4	58	10	4	16	—
5	58	11	2	17	1
6	40	12	—		

Average 4.8 eggs per an egg-mass.

Host Plants on Which the Female Fly Deposits Her Eggs

Approximately thirty species of plants have been recorded as hosts of this fly. These plants are distributed in six families, viz., Chenopodiaceae, Solanaceae, Caryophyllaceae, Polygonaceae, Compositae and Rosaceae. The fly has been found to oviposit on six species of plants in Korea and Japan, namely *Atriplex subcordata* var. *japonica* (Koidzumi), *Beta vulgaris* Linné, *Beta vulgaris* var. *Rapacea* C. Koch, *Chenopodium centrorubrum* Nakai, *Kochia scoparia* Schrader and *Spinacia oleracea* Linné. Among the races of *Spinacia* such races as *Ujo, Japanese Kairyooba, Jiromaru, Long Standing, Viroflay, Hollandia* and *Munsterland* have been observed to be oviposited and infested. So far as the authors observations go, the fly does not oviposit on the leaves of the Japanese race of spinach. Such races mentioned above are either European, Chinese or hybrids between those and the Japanese races. On April 28th, 1945, a single egg-mass of the fly was found on the leaf of *Commelina communis* Linné (Commelinaceae) in the spinach field, but the larvae could not continue their normal growth and all died 8 days after hatching. Thus in Northern Kyushu the fly oviposits exclusively on the plants belonging to the family Chenopodiaceae.

As to the relation of *Pegomyia hyoscyami* to its host plants, Cameron (1914, 1916) gave interesting studies. In this connection the authors made the following three experiments, the results of which are summarized in Tables 4, 5 and 6.

Experiment 1: To determine whether adults of *Pegomyia hyoscyami*, reared from larvae which had fed on the leaves of *Chenopodium centrorubrum*, would oviposit mainly on the same plant, about 18 mated females were confined in a breeding cage containing potted plants of *Chenopodium centrorubrum*, *Atriplex subcordata* var. *japonica* and the Japanese race of *Spinacia oleracea*.

Table 4. Number of eggs deposited by *Pegomyia hyoscyami* females reared from *Chenopodium centrorubrum*.

Date of oviposition	*Chenopodium centrorubrum*	*Atriplex subcordata* var. *japonica*	Japanese race of spinach
22. v.	9	6	0
23. v.	18	20	0

24. v.	11	11	0
26. v.	13	14	0
27. v.	0	4	0
30. v.	0	1	0
31. v.	4	2	0
Total	55	58	0

The differences in the number of eggs deposited on three different plants are significant ($P < 0.01$). *Pegomyia hyoscyami* reared on *Chenopodium centrorubrum* did not oviposit on the Japanese race of spinach.

Experiment 2: To determine whether adults of *Pegomyia hyoscyami*, reared from larvae which had fed on the leaves of *Atriplex subcordata* var. *japonica*, would oviposit chiefly on the same plant, about 36 mated females were confined in a breeding cage containing potted plants of *Atriplex subcordata* var. *japonica*, *Chenopodium centrorubrum* and an European race of spinach, *Viroflay*.

Table 5. Number of eggs deposited by *Pegomyia hyoscyami* females reared from *Atriplex subcordata* var. *japonica*.

Date of oviposition	*Atriplex subcordata* var. *japonica*	*Chenopodium centrorubrum*	Spinach, *Viroflay*
22. v.	11	7	0
23. v.	14	19	0
24. v.	35	14	0
25. v.	29	13	1
26. v.	34	10	0
27. v.	3	6	0
28. v.	0	10	0
29. v.	2	12	3
30. v.	9	10	0
31. v.	0	4	0
1. vi.	0	3	0
Total	137	108	4

The differences in the number of eggs deposited on three different plants are highly significant ($P < 0.05$).

Experiment 3: To determine whether adult *Pegomyia hyoscyami*, reared from larvae which had fed on the leaves of an European race of spinach, *Viroflay*, would oviposit chiefly on the same plant, about 31 mated females were confined in a breeding cage containing potted plants of *Viroflay*, the Japanese race of spinach and *Chenopodium centrorubrum*.

Table 6. Number of eggs deposited by *Pegomyia hyoscyami* females reared from *Viroflay*.

Date of oviposition	Spinach, *Viroflay*	Japanese race of spinach	*Chenopodium centrorubrum*
25. v.	0	0	6
26. v.	5	1	8
27. v.	6	0	14
28. v.	0	0	10
29. v.	4	0	3
30. v.	0	0	8
1. vi.	2	0	0
Total	17	1	49

The differences in the number of eggs deposited on three different plants are highly significant ($P < 0.05$). The deposition of one egg on the Japanese race of spinach would be accidental.

As can be seen from Tables 4, 5 and 6, the order of egg-laying preference for *Pegomyia hyoscyami* females is; *Atriplex subcordata* var. *japonica* > *Chenopodium centrorubrum* > European race of spinach, *Viroflay*. Further it is evident that the fly does not oviposit on the Japanese race of spinach at all or the larvae of this fly cannot complete their life-history in the Japanese spinach. This fact would indicate that the females will not oviposit on the Japanese race of spinach even if host plants are entirely absent in the field.

Reason of Recent Increase in Population of *Pegomyia hyoscyami* in Northern Kyushu

Observations of the dates of adult emergence of this fly from both field-collected and laboratory-reared puparia indicated that in 1944 adults of the overwintered generation emerged during April

to the beginning of May, and those of the first generation during the middle to end of May and at the beginning of June and the second generation (Table 7, A) during October to the beginning of November, though some puparia of this generation overwintered and gave rise to adults in the following April May (Table 7, B). In April and May adult flies can easily find the native host plants for oviposition, viz. *Atriplex subcordata* var. *japonica* and *Chenopodium centrorubrum*, but in the autumn those plants are not available in the field. Therefore flies emerged in September and October cannot oviposit their mature eggs. Thus it is presumably only these overwintered puparia of the second generation that give rise to the spring adults. These relation may easily be recognizable by Table 7.

Table 7. Life-history of *Pegomyia hyoscyami* in Northern Kyushu, 1945–1946. ●: eggs, −: larvae, +: pupae, ○: adults.

As indicated in the introduction of this paper, *Pegomyia hyoscyami* has become increasingly troublesome throughout the spinach growing areas of Northern Kyushu since 1939. Namely some time before that year the cultivation of some European and Chinese races or hybrids between European and Japanese races of spinaches was begun. Such spinaches were cultivated in the field from October to the following May. Thus adult flies became to oviposit their eggs both in the spring and autumn.

This is the reason why populations of this fly have become increased markedly since 1939.

Distribution of Eggs in the Spinach Field

In market-garden areas in Fukuoka Prefecture it is the custom to plant spinach in long, comparatively narrow strips. In order to obtain informations on the distribution of *Pegomyia* eggs, four widely separated areas of spinach were selected. In all instances where this was observed, however, the crops examined were uniform as regards the condition of the plants. Area No. 1 was subdivided into 45 subdivisions of equal size, likewise No. 2 into 25, No. 3 into 30 and No. 4 into 20 subdivisions respectively. Records of the egg populations on each subdivision were kept and in this way the distribution of eggs could be investigated. The results are shown in Tables 8, 9, 10 and 11.

Table 8. Distribution of eggs of *Pegomyia hyoscyami* in the spinach field No. 1 (Observation made in the autumn of 1946).

Division of each strip	Strip No.									Total no. of eggs
	1	2	3	4	5	6	7	8	9	
1	3	0	4	3	5	2	4	8	4	33
2	3	2	4	9	1	9	0	5	5	38
3	19	5	7	7	10	20	2	2	10	82
4	9	8	8	3	7	7	4	8	6	59
5	4	14	7	8	6	2	0	12	17	70
Total no. of eggs	38	29	30	30	29	40	10	35	41	282

Table 9. Distribution of eggs in the spinach field No. 2.

Division of each strip	Strip No.					Total no. of eggs
	1	2	3	4	5	
1	35	76	52	33	33	229
2	92	99	32	37	31	291
3	74	126	31	65	61	357
4	50	68	40	37	75	270
5	59	84	33	83	57	316
Total no. of eggs	310	453	188	255	257	1473

Table 10. Distribution of eggs in the spinach field No. 3.

Division of each strip	Strip No.						Total no. of eggs
	1	2	3	4	5	6	
1	32	7	20	0	0	0	59
2	0	8	8	1	8	13	38
3	0	1	0	0	0	19	20
4	16	22	6	1	9	27	81
5	6	11	6	14	25	10	72
Total no. of eggs	54	49	40	16	42	69	270

Table 11. Distribution of eggs in the spinach field No. 4.

Division of each strip	Strip No.				Total no. of eggs
	1	2	3	4	
1	91	30	23	6	150
2	106	12	60	23	201
3	50	3	53	9	115
4	50	0	32	34	116
5	80	8	114	32	234
Total no. of eggs	377	53	282	104	816

The difference in the number of eggs between the subdivisions is either likely significant or insignificant. It is, therefore, clear that there is no evidence of any tendency of the distribution of *Pegomyia* eggs in the spinach area. In other words, the adults neither prefer nor select the border or inside of spinach area in depositing their eggs. This fact is most likely to be ascribed to a permanent source of infestation which exists internally to the areas themselves.

Relation Between the Time of Sowing of Spinach and the Number of Eggs Laid

As to the relation between the time of sowing of spinach and the number of eggs laid by *Pegomyia hyoscyami*, the authors give summaries of observations made by several entomologists in the following lines.

Wilke (1922) in Germany :—Early sown beet seems to suffer more than late-sown.

Kleine (1933) in Germany :—The correctness of the practice of late sowing, which is often adopted, is confirmed.

Blunck (1925) in Germany :—Instead of late sowing, advised by Kleine, the author suggests early sowing, so that the plants may not be attacked when yet tender seedlings.

Blunck (1927) in Germany :—Although it is true that late-sown beet remains free from attack by the beet-fly, it is necessary to sow early in North Germany, owing to the losses in weight and sugar-content consequent on late sowing.

Rambousek and Neuwirth (1931) in Czechoslovakia : In two adjoining fields beet sown late escaped injury, whereas that sown early was severely infested.

Roebuck, Baker and White (1945) in England :—Infestation by the first generation was avoided if sowing was delayed until mid May in 1945, such late-sown crops were not attacked to any extent by the later generations.

In order to ascertain whether such relation may exist also in Northern Kyushu, the following field experiment was undertaken. In the spring of 1947 seeds of *Viroflay* were sown side by side in four narrow strips in four different dates (March 15, March 25, April 4 and April 14) before emergence of adult flies of the over-wintered generation, and the date and number of eggs laid by *Pegomyia hyoscyami* were recorded. The results are shown in Table 12.

Table 12. Number of eggs of *Pegomyia hyoscyami* deposited on four groups of spinach plants which were sown in narrow strips side by side at different dates in 1947.

Date of oviposition	Date of sowing			
	15. iii.	25. iii.	4. iv.	14. iv.
26. iv.	13 eggs	0	0	0
27. iv.	19	0	0	0
28. iv.	18	0	0	0
29. iv.	14	0	0	0
30. iv.	0	9	0	0
1. v.	0	1	12	0

3. v.	0	4	3	0
4. v.	2	14	3	0
5. v.	0	6	0	0
6. v.	0	3	0	0
8. v.	3	0	2	0
12. v.	2	0	12	0
14. v.	0	4	0	2
Total no. of eggs	71	51	22	2

Thus the results have clearly shown that the number of eggs deposited on the leaves of spinach by the adult flies depended upon the time of sowing of its seeds. Attention must here be called that the larger the leaves the more the eggs or the younger the plants the fewer the eggs.

Résumé

Hitherto not a single detailed observation on the egg-laying habits of *Pegomyia hyoscyami* Panzer has been performed in Japan. The present investigations have been carried out near and in the campus of the Kyushu University, Fukuoka, Northern Kyushu, during 1943 1947. The results are set out in nine chapters given above. The most important conclusion to be derived from the authors' investigations is the fact that the fly does not oviposit on the leaves of the Japanese race of spinach and the cultivation of some European, Chinese or hybrids between those and the Japanese races initiates a marked increase in its population. Some bearing of the results on measures for controlling this fly will be evident to any one who reads the contents.

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Journal of the Faculty of Agriculture, Kyūshū University, Vol. 10, No. 2
September 30, 1951

A NEW SYSTEM OF CLASSIFICATION OF THE GENUS *DEBARYOMYCES* BASED ON ACID PRODUCTION

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Yeasts belonging to the genus *Debaryomyces* play an important role in the preservation of food. They are different from other yeasts ecologically because of their wide distribution and high salt tolerance, and especially because of their common occurrence in brined, pickled, and other meat products.[1] On the other hand, they differ physiologically from the other true yeasts in the production of flavin pigment.[2] Although they are of much interest in this respect, their usefulness has not been recognized in the fermentation industry.

Among the taxonomic studies of *Debaryomyces*, which have hitherto been made, systems have been devised by Césari & Guilliermond (1920),[3] Konokotina & Krassilnikov (1929),[4] Stelling-Dekker (1931),[5] Lodder (1934),[6] and in the field of medicine, by Dodge (1935)[7]; and recently another new system was presented by Naganishi (1948).[8]

The ability of sugar-fermentation has been generally employed as an important key in classifying the true yeasts. But, the fermentation ability of all species belonging to *Debaryomyces*, with a few exceptions, is unstable and variable, as already pointed out by Naganishi (1940).[9] Therefore, the classification of *Debaryomyces* presented by Konokotina & Krassilinikov, Stelling-Dekker, Lodder, and Dodge, based on sugar-fermentation, cannot be considered applicable. Naganishi, in his system, while dividing *Debaryomyces* into "yellowish strain group"* and "non-yellowish strain group",

* The species belonging to this group produce a lemon-yellow pigment on the modified Hayduck medium and change the color of the medium to yellow, glimmering with green fluorescence, whereas the other group produces no yellow pigment on the same medium, and is generally fermentative.

did not employ the sugar-fermentation test for the identification of species in the former group.

As regards the specific characteristics of *Debaryomyces*, Zopf (1889)[10] reported the production of oxalic acid, and the same fact was demonstrated by Perwozwansky (1930).[11] The species used in their studies, *Saccharomyces Hansenii*, was amended to *Debaryomyces tyrocola* var. *Hansenii* (Zopf) Dekker. Naganishi (1940)[12] estimated the acidity of Koji water cultures using a large number of the species of this genus.

Taking these facts into consideration, the author investigated the ability of yeasts to produce acid from various kinds of sugar, and found it useful in the classification of *Debaryomyces*. The acid production test has been applied widely in the classification of bacteria, though never to that of true yeast. However, in the case of asporogenous yeast, the applicability of the test was ascertained by Castellani (1913),[13] who applied it to the genus *Monilia*; and Martin, et al. (1940),[14] to the genus *Candida*. Strong acid production by the genus *Brettanomyces* is also given considerable taxonomic weight by Custer (1940).[15]

In this paper, a new classification system of *Debaryomyces* based on acid production will be presented, together with the description, not only of its action on several sugars which have been previously tested in order to be employed for the identification of species or group, but also of the experimental conditions about "standard test" in detecting acid production.

Strains of *Debaryomyces* Used in the Experiment

Of the 31 strains used in this study, 18 were isolated and identified by the author, while undetermined strains are merely described as *Debaryomyces* sp. The yeasts that were tested, are listed in the following table.

Table 1. Strains of *Debaryomyces* used.

1. *Debaryomyces membranaefaciens* Naganishi.
2. *Debaryomyces membranaefaciens* var. *Zingiberi* Otani.
3. *Debaryomyces membranaefaciens* var. hollandicus Lodder.
4. *Debaryomyces* sp. C_2 (*D. membranaefaciens* type).
5. *Debaryomyces* sp. O_9 (*D. membranaefaciens*).
6. *Debaryomyces* sp. P_2 (*D. tyrocola* type).

7. *Debaryomyces japonicus* Naganishi.
8. *Debaryomyces* sp. C_4 (*D. tyrocola* type).
9. *Debaryomyces* sp. S_3 („ „).
10. *Debaryomyces* sp. C_1 („ „).
11. *Debaryomyces* sp. Q_3 („ „).
12. *Debaryomyces* sp. Q_4 („ „).
13. *Debaryomyces miso* Mogi.
14. *Debaryomyces miso* var. *α* Mogi.
15. *Debaryomyces* sp. Y_1.
16. *Debaryomyces Klöckeri* Guilliermond et Péju.
17. *Debaryomyces* sp. S_4 (*D. Klöckeri* type).
18. *Debaryomyces* sp. A_2 (*D. Matruchoti* type).
19. *Debaryomyces manchuricus* Naganishi.
20. *Debaryomyces globosus* Klöcker.
21. *Debaryomyces* sp. V_1.
22. *Debaryomyces* sp. X_3.
23. *Debaryomyces sake* Saito et Oda.
24. *Debaryomyces Matruchoti* Grigoraki et Péju.
25. *Debaryomyces* sp. S_2 (D. n. sp. unpublished)
26. *Mycoderma* sp. Q. Naganishi evaluated this yeast as *Debaryomyces*.
27. *Debaryomyces* sp. W_1.
28. *Debaryomyces tyrocola* var. *Hansenii* (Zopf) Dekker.
29. *Debaryomyces orientalis* Naganishi.
30. *Debaryomyces* sp. R_4.
31. *Debaryomyces Guilliermondii* var. *nova-zeelandicus* Lodder.

Action of *Debaryomyces* on Sugars

a) *Fermentation test*:—As shown in the classification of Stelling-Dekker and of Lodder, there are many species which have no fermentation ability. According to Naganishi (1940), they are found to have the ability, in the case of fresh yeast cells, and a number of notices have been given by him about the procedure of the fermentation test. The author has frequently experienced that, even with fresh cells, there occurs no fermentation, or, if it does, it is very erratic. Therefore, the fermentation reaction of *Debaryomyces* (excepting the fermentative group by Naganishi) is considered as insufficient for the basis of classification.

b) *Sugar-assimilation test*:—Lodder[16] has applied the assimilability of sugar as a key to classifying non-fermentative species, which belong to *Torulopsidaceae*, and furthermore, Diddens & Lodder (1942)[17] have extended its employment to all species of *Mycotoruloideae*. The author made an investigation to determine whether this test could be adopted as a key to classifying

Debaryomyces. Naganishi (1934)[15] studied the ability of sugar-assimilation with this genus, using the modified Hayduck's medium. The author repeated a similar test on some selected species using Lodder's medium. The basal medium consists of 0.5 % $(NH_4)_2SO_4$, 0.1 % KH_2PO_4, 0.01 % $MgSO_4 \cdot 7H_2O$. To this solution 2 % each of the test sugars were added. Each 5 cc. of the medium was inoculated with two drops of yeast suspension in sterilized water, and the cultures were examined after five and ten days at 25°C. The results are shown in Table 2.

Table 2. Sugar-assimilation test of several species.

Strains \ Sugar	glucose	fructose	mannose	galactose	sucrose	maltose	lactose	raffinose	xylose	arabinose	rhamnose
D. sake	+	+	++	++	++	++	−	+	+	++	
D. miso	++	++	++	++	++	++	−	+	+	++	−
D. Klöckeri	+	+	+	++	+	++	−	+	+	++	−
D. orientalis	+	+	+	+	+	+	+	+	+	++	−
D. japonicus	++	++	+	+	++	+	−	++	+	++	−
D. sp. R_4	+	+	+	+	+	+	+	+	+	+	−
D. sp. C_2	++	++	++	++	++	++	−	+	+	++	−
D. sp. Q_3	+	+	+	+	+	+	−	+	+	++	−
D. sp. S_3	++	++	++	++	++	++	−	+	+	++	−
D. sp. C_1	++	+	++	++	++	++	−	+	+	+	−

No remarkable differences were found in their ability to assimilate the sugar, with the exception of lactose, although the test yeasts showed an obvious difference from ordinary yeasts in the case of pentose-assimilation. Thus, the sugar assimilability is considered to be of no significance in the taxonomy of this genus, with the exception that lactose-assimilation seems to be helpful in some cases.

c) *Acid production test*:—The test methed was established as follows:

On Acid Production of *Debaryomyces*

a) ***Relation between pH and acid production.***

In establishing the standard test by detecting acid-production,

it is necessary to know the relation between acidity and pH during the culture of *Debaryomyces*. Accordingly, the author experimented with typical species of this genus for acid-producing ability, fate, and pH change.

The test yeast: The fermentable species—*D. manchuricus* Naganishi and *D. globosus* Klöcker—and the unfermentable species—*D.* sp. P_2, *D.* sp. S_4, and *D. tyrocola* var. *Hansenii*—were used.

The experimental procedure: The basal medium was prepared as follows: 5 % glucose, 0.25 % peptone, and 1.0 % NaCl were dissolved in distilled water, and the pH was adjusted at 7.0. 90 cc. of basal medium in a 100 cc. Erlenmeyer flask was inoculated with the test yeast, cultured on Sabouraud agar at 25 C for a few days, and allowed to stand at 25 C. The acidity of 5 or 10 cc. of the sample was estimated by titrating with 0.01N NaOH, using phenolphthalein as an indicator. The acidity is shown in the following table by the number of cc. of 0.1N NaOH necessary to neutralize the acid in 100 cc. of medium. The pH value was measured electrometrically.

The results obtained: Both of the fermentable species were strong acid producers. Acidity-curves distinguished them from the unfermentable species (Fig. 1), and a similar tendency was observed in the pH curves (Fig. 2). Among the species of each group, there

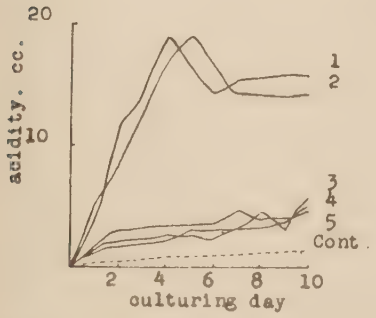

Figure 1. Change of acidity in the culture of some species of *Debaryomyces*.

1. *Debaryomyces globosus*.
2. *Debaryomyces manchuricus*.
3. *Debaryomyces* sp. S_4.
4. *Debaryomyces* sp. P_2.
5. *Debaryomyces tyrocola* var. *Hansenii*.

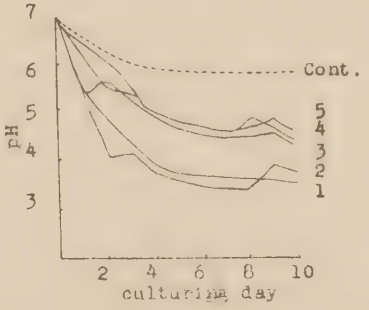

Figure 2. Change of pH by acid production of some species of *Debaryomyces*.

is little difference in acidity- and pH-curves. Since the pH value of the culture medium exhibits a tendency to decrease below a pH of 5.0 within 2–4 days, its degree of change will be available for the detection of acid production.

b) *Relation between the acid-producing ability and its pH in various concentrations of sugar.*

This experiment was carried out to decide a sugar concentration strong enough to have a distinct effect on the indicator, though most of *Debaryomyces* do not need as great a quantity of sugar as the alcohol yeasts as a source of energy.

The test yeast: Since the previous experiment showed no marked difference among the strains in each group, the following two typical species, *D. globosus* Klöcker and *D.* sp. C_2, were used.

The experimental procedure: The concentration of glucose solutions used, were 0.5, 1.0, 2.0, 3.0, and 5.0%, respectively. All other conditions were the same as in the previous experiment.

The results obtained: *D. globosus* showed the maximum acidity within 2–4 days. The acidity then decreased gradually (Fig. 3), and the pH dropped below 5.0 (Fig. 4). In the case of *D.* sp. C_2, the acidity gradually increased, and the pH decreased independently of sugar concentration (Fig. 5). Hence, 0.5–2.0% sugar solution is considered to be adequate.

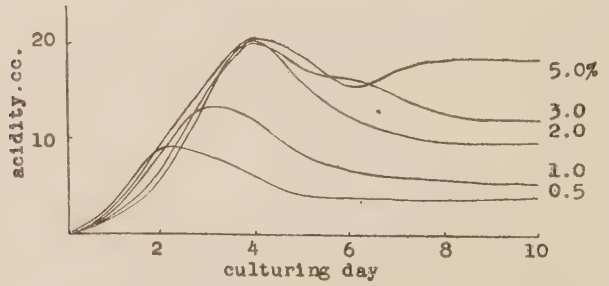

Figure 3. Change of acidity in the cultures of *D. globosus* with various concentrations of glucose.

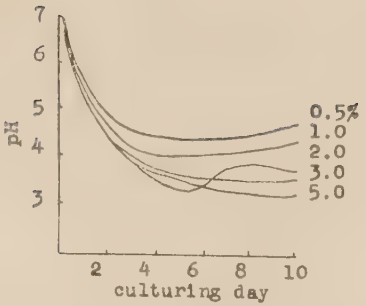

Figure 4. pH change in the cultures of *D. globosus* with various concentrations of glucose.

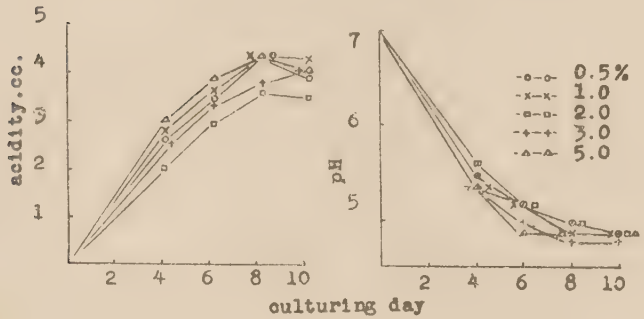

Figure 5. Changes of pH and acidity in the cultures of *D.* sp. C_2 (*D. membranaefaciens* type) with various concentrations of glucose.

c) *Establishment of standard test in acid production.*

Basal medium for standard test: Barsiekow's medium is usually used for the acid production test on bacteria, but it is unsuitable for the identification of yeast. The test medium for this purpose requires a suitable ratio between nitrogen and carbone; and addition of nutrient salts, such as phosphates, exhibits a tendency to delay acid production. Therefore, the basal medium was prepared as follows: 0.25 % peptone, 1.0 % NaCl, and 2 % test sugar were dissolved in distilled water, and 0.2 % Bromthymol blue solution was added to facilitate the judgment of acid production (12 cc. of the indicator to 1 liter of basal medium). The pH was then adjusted at 7.0. 3 to 4 cc. of the medium were placed in a small test tube and sterilized by ordinary procedure.

Experimental procedure: In general, American-type Sabouraud agar was used as the pre-culture medium. No difference from wort agar was perceived during the course of this experiment. During the incubation period, two day- and five day-cultures gave a consistent result with a slight difference in detecting-days. The results of comparison between the author's method with that of Martin (Table 3) showed that there was a tendency for delayed acid production in the Martin's method.

Table 3. Comparison of acid production test between Martin's method and the author's.

Sugars \ Strains		*D. sake*						*D.* sp. R_4							*D.* sp. Q_3						
		1	2	3	4	5	6	1	2	3	4	5	6	7	1	2	3	4	5	6	7 days
Glucose	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	M	−	+	+	+	+	+	−	+	+	+	+	+	+	−	+	ǀ	ǀ	ǀ	+	ǀ
Maltose	A	−	−	−	−	−	−	−	−	±	+	+	+	+	−	−	±	±	±	±	+
	M	−	−	−	−	−	−	−	−	−	±	+	+	+	−	−	−	+	+	+	+
Sucrose	A	−	+	+	+	+	+	±	+	+	+	+	+	+	−	±	+	+	+	+	+
	M	−	−	±	±	+	+	−	−	±	+	+	+	+	−	−	±	+	+	+	+
Lactose	A	−	−	−	−	−	−	−	−	±	±	+	+	+	−	−	−	−	−	−	−
	M	−	−	−	−	−	−	−	−	−	−	−	±	±	−	−	−	−	−	−	−

Note: A−The author's method, M−Martin's method.

Color and pH *change in basal medium*: In order to determine a color and pH change during the cultivation, the relation between them was observed on 0.25% peptone water over a wide range of pH. The relations are as follows:

Color	pH	Degree of acidity
lemon yellow	below 5.4	ǂǂ
slight greenish-yellow	ca. 5.4-5.5	ǂ
light green	ca. 5.6-5.8	+
light blue	above 5.9	−

d) *Acid production test with various kinds of sugar.*

On 31 strains of this genus, the acid production test was carried out by the standard method, using glucose, maltose, sucrose, lactose, raffinose and galactose, with the following results (Table 4).

Table 4. The results of acid production test.

*	*D. tyrocola* var. *Hansenii* 1	2	3	4	5	6 day	*D. Guilliermondii* var. *nova-zeelandicus* 1	2	3	4	5	6	*D. orientalis* 1	2	3	4	5	6
D	−						−	+										
M		−	−				−	−	−				−	−	−			
S	−						−											
L	−	−						−	−	−			−	−	−			
R	−	−					−	−								−		
G													−	+				

	D. sp. R_4 1	2	3	4	5	6	*D. membranaefaciens* 1	2	3	4	5	6	*D. membranaefaciens* var. *Zingiberi* 1	2	3	4	5	6
D																		
M	−	−					−						−	−				
S	−						−						−	−				
L	−	−					−	−	−						−	−		
R															−			
G	−						−						−					

	D. membranaefaciens var. *hollandicus* 1	2	3	4	5	6	*D.* sp. O_9 1	2	3	4	5	6	*D.* sp. C_2 1	2	3	4	5	6
D	−																	
M	−	−	+				−	+					+					
S	−																	
L		−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−
R		−					+						−	+				
G																		

	D. miso 1	2	3	4	5	6	*D. miso* var. *α* 1	2	3	4	5	6	*D. japonica* 1	2	3	4	5	6
D							−											
M							−	−										
S	−						−											
L		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
R	−						−	−	+				−	+				
G							−											

	D. sp. C_1 1	2	3	4	5	6	*D.* sp. C_4 1	2	3	4	5	6	*D.* sp. S_3 1	2	3	4	5	6
D	+																	
M	−	−	−	−			−	−					−					
S	+																	
L	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
R	−	−	−	+			−	−					−	−				
G							+						+					

* Note: The test sugar.

D. – glucose S. – sucrose R. – raffinose

M. – maltose L. – lactose G. – galactose

D. sp. P_2

	1	2	3	4	5	6
D	++	+	++	++	++	++
M	+	+	+	++	++	++
S	++	++	++	++	++	++
L	–	–	–	–	–	–
R	+	++	++	++	++	++
G	+	++	++	++	++	++

D. sp Q_4

	1	2	3	4	5	6
D	+	++	++	++	++	++
M	–	+	+	+	+	++
S		–	+	++	++	++
L	–	–	–	–	–	–
R	–	–	–	·	+	+
G	+	++	++	++	++	++

D. Klöckeri

	1	2	3	4	5	6
D	++	++	++	++	++	++
M	–	–	–	–	++	++
S	+	++	++	++	++	++
L	–	–	–	–	–	–
R	–	–	–	–	+	++
G	++	++	++	++	++	++

D. sp. V_1

	1	2	3	4	5	6
D	–	–	+	+	++	++
M	–	–	–	–	+	++
S	–	–	+	++	++	++
L	–	–	–	–	–	–
R	–	–	–	–	–	–
G	+	+	+	+	++	++

D. sake

	1	2	3	4	5	6
D	–	++	++	++	++	++
M	–	–	–	–	–	–
S	–	–	–	++	++	++
L	–	–	–	–		–
R	–	–	–	–	–	–
G	–	+	++	++	+	+

D. sp. W_1

	1	2	3	4	5	6
D	–	+	++	++	++	++
M	–	–	–	–	–	–
S	–	–	–	–	–	–
L	–	–	–	–	·	–
R	–	–	–	–	–	–
G	–	–	–	+	++	++

D. sp. Y_1

	1	2	3	4	5	6
D	+	++	++	++	++	++
M	–	–	–	++	++	++
S		+	++	++	++	++
L	·	·	–	–	–	–
R	–	–	–	–	–	+
G	–	++	++	++	++	++

D. manchuricus

	1	2	3	4	5	6
D	++	++	++	++	++	++
M	·	–	++	++	++	++
S	++	++	++	++	++	++
L	·	–	–	–	·	–
R	·	–	++	++	·	++
G	–	++	++	++	++	++

D. sp. S_4

	1	2	3	4	5	6
D	+	++	++	++	++	++
M	–	+	++	++	++	++
S	+	+	++	++	++	++
L	–	–	–	–	–	–
R	–	+	++	++	++	++
G	+	++	++	++	++	++

D. sp. X_3

	1	2	3	4	5	6
D	+	++	++	++	++	++
M	–	–	+	++	++	++
S	–	+	+	+	++	++
L	–	–	–	–	–	–
R	–	–	–	–	–	+
G	+	+	+	++	++	++

Mycoderma sp. Q

	1	2	3	4	5	6
D	+	++	++	++	++	++
M	–	–	–	–	–	–
S	–	·	+	+	++	++
L	–	–	–	–	·	–
R	–	·	–	–	·	–
G	–	–	+	+	+	+

D. sp. Q_3

	1	2	3	4	5	6
D	++	++	++	++	++	++
M	–	–	+	++	++	++
S	–	–	+	++	++	++
L	–	–	–	–	–	–
R	–	–	–	+	+	+
G	+	++	++	++	++	++

D. globosus

	1	2	3	4	5	6
D	++	++	++	++	++	++
M	–	–	++	++	++	++
S	++	++	++	++	++	++
L	–	–	–	–	–	–
R	++	++	++	++	++	++
G	–	–	–	–	–	–

D. sp. A_2

	1	2	3	4	5	6
D	+	++	++	++	++	++
M	–	–	–	+	++	++
S	·	+	++	++	++	++
L	·	–	–	–	–	–
R	–	–	+	++	++	++
G	+	+	++	++	++	++

D. Matruchoti

	1	2	3	4	5	6
D	–	++	++	++	++	++
M	·	–	–	–	–	–
S	·	–	–	–	–	+
L	–	–	–	–	–	–
R	·	–	·	–	–	+
G	–	–	+	+	+	+

D. sp. S_2

	1	2	3	4	5	6
D	+	++	++	++	++	++
M	–	–	–	–	–	–
S	–	+	++	++	++	++
L	–	–	–	–	–	–
R	–	+	++	++	++	++
G	–	–	–	–	–	–

A new Classification System for *Debaryomyces*

The 31 strains tested can be divided into the following four groups, according to their ability to produce acid from sugar.

I. Acid from glucose, sucrose, maltose, and lactose .....4 strains.
II. Acid from glucose, sucrose, and maltose .....22 strains.
III. Acid from glucose and sucrose..... 4 strains.
IV. Acid from glucose ..... 1 strain.

Considering the results of the acid production test, using raffinose and galactose, and of the film formation test by Stelling-Dekker with the above groups, the author presents a new classification system for *Debaryomyces* as follows:

Group I. Acid from glucose, sucrose, maltose, and lactose.

Debaryomyces tyrocola var. *Hansenii* (Zopf) Dekker
D. Guilliermondii var. *nova-zeelandicus* Lodder
D. orientalis Naganishi
D. sp. R_1

Group II. Acid from glucose, sucrose, and maltose.

a) Film-formation on wort.
b) Greyish-white folded film.

D. membranaefaciens Naganishi
D. „ var. *Zingiberi* Otani
D. „ var. *hollandicus* Lodder
D. sp. O_9 (*D. membranaefaciens*)
D. sp. C_2 (*D. membranaefaciens* type)

bb) Grey thin film.
c) Acid from raffinose.

D. miso Mogi
D. miso var. α Mogi
D. japonicus Naganishi
D. sp. C_1 (*D. tyrocola* type)
D. sp. C_4 („ „)
D. sp. S_3 („ „)
D. sp. P_2 („ „)

cc) Acid from raffinose weakly.

D. sp. Y_1 (*D. tyrocola* type)
D. sp. Q_3 („ „)

D. sp. Q_4 („ „)

aa) No film on wort.

b) Gas and acid from glucose.

c) Acid from galactose.

D. manchuricus Naganishi

cc) No acid from galactose.

D. globosus Klöcker.

bb) No gas from glucose.

c) Acid from raffinose.

D. Klöckeri Guilliermond et Péju

D. sp. S_4 (*D. Klöckeri* type)

D. sp. A_2 (*D. Matruchoti* type)

cc) No, or less acid from raffinose.

D. sp. V_1

D. sp. X_1

Group III. Acid from glucose and sucrose.

a) Acid from galactose.

b) Acid from raffinose.

D. Matruchoti

bb) No acid from raffinose.

D. sake Saito et Oda

Mycoderma sp. Q

aa) No acid from galactose.

D. sp. S_2 (*D.* n. sp. unpublished)

Group IV. Acid from glucose.

D. sp. W_1

Discussion

The routine fermentation test is not of great singnificance in the case of *Debaryomyces*, but the acid production test is worthy of consideration as an important factor in differentiating the species of this genus. In employing this test it is necessary to decide the number of culturing-days required to judge the ability of acid production. Because of the following facts, the author considers it suitable to adopt the results of six culturing days:

1) In some rapid cases, acid production was observed within 24 hours, and it could be detected within 4–6 days even in the slow acid-producers; 2) when the test culture is allowed to incubate for a prolonged period, the color change becomes obscure; 3) a few species sometimes display a tendency to revert to the initial color after once changing; 4) the color of the medium itself may change during a long incubation.

As pointed out by a few workers, intermediate strains between *D. membranaefaciens* type and *D. Guilliermondii* type are very difficult to separate by the state of the film, whereas by the acid production from lactose, both types are easily distinguishable. *D. Guilliermondii* strain K, *D.* sp. H (*D. Guilliermondii* strain ?), *D. Guilliermondii* Dekker, and some of the other strains will probably be qualified for Group I, because Naganishi's test indicated that they were capable of assimilating lactose. This assimilability was exhibited by 4 of the 13 strains representing *D. tyrocola*, though by the author's test, all strains of this type that were tested were unable to produce acid from lactose and belonged to Group II.

This system is considered to be applicable for the taxonomy of *Pichia* and *Zygopichia*, having oxidative or weak fermentative ability, since the new system, based on acid production, gave simple and distinct diagnoses of *Debaryomyces*. It will become more useful by adopting the utilization test of nitrite and urethan (Naganishi 1948), and the growth test at moderate osmotic pressure (Wickerham 1951).

Summary

A standard test detecting the acid production for taxonomic studies of *Debaryomyces* was devised, and the acid-producing ability was investigated on 31 strains.

The yeasts tested, were divided into 4 groups, according to their ability of producing acid from glucose, maltose, sucrose, and lactose. Moreover, they were classified by the acid production test with raffinose and galactose, as well as by the state of the film. Thus a new classification system of *Debaryomyces* has been proposed.

Acknowledgment

This investigation was completed at the Chemical Laboratory of Aquatic Products, Faculty of Agriculture, Kyushu University, under the direction of Professor Yukio Tomiyasu. The author wishes to express sincere gratitude for his kind guidance during the course of the investigation.

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(The mark* indicates an essay which was published in Japanese).

Journal of the Faculty of Agriculture, Kyūshū University, Vol. 10, No. 2
September 30, 1953

ON THREE NEW SPECIES OF *GALATHEA* FROM THE WESTERN PACIFIC[1]

SADAYOSHI MIYAKE

The Galatheids dealt with in this paper are all preserved in the collection of the Zoological Laboratory, Faculty of Agriculture, Kyushu University. I wish to express my hearty thanks to Prof. Dr. Y. K. Hiraiwa, Zoological Laboratory, Faculty of Agriculture, Kyushu University, who has provided me with facilities required for the pursuit of this work. I am especially indebted to Dr. H. Ohshima, M. J. A., for giving me the opportunity to study the material collected in his expeditions.

1. *Galathea biunguiculata* sp. nov.
Text-fig. 1–2.

The carapace is rather depressed and the upper surface is furnished with squamiform scales beneath tomentum. The rostrum is equipped with four teeth on each lateral margin. Lateral margins of the carapace are equipped with six teeth: the first tooth is smallest of all; the second is largest; succeeding ones decrease in size posteriorly. There are two small teeth on the gastric region.

The sternum of the third maxilliped is very broad, of a triangular shape, with a small but prominent sinus at the tip. The sternum of chelipeds diverges posteriorly, its lateral margins being almost straight. The first peduncle of the antennule bears three spines: the inner and the outer spines are furnished with long hairs; the second peduncle of the antenna bears an acuminate spine on each inner and outer angle; the merus of the third

[1] Contributions from the Zoological Laboratory, Faculty of Agriculture, Kyushu University, No. 207.

maxilliped is equipped with two teeth on the inner margin, but is toothless on the outer margin.

The chelipeds are slightly depressed, 1.5 times as long as the length of carapace and furnished with long hairs along the inner

Fig. 1. *Galathea biunguiculata* sp. nov.
Holotype (ovigerous ♀) from Palau Islands, ×10.

and outer margins. The arm is provided with two prominent teeth on inner distal end and a row of five spines runs on the upper surface. There is a prominent tooth at the outer extremity of the arm. The wrist is a little shorter than half the palm, the inner margin being equipped with four teeth, the outer margin

with three. The palm is broad, provided with five spines near the inner margin; in addition to them there are eight teeth on the outer margin. Fingers are furnished with small teeth and pubescent on cutting margins.

The ambulatory legs are depressed, being furnished with long hairs along the anterior and posterior margins. The merus is furnished with seven to nine spines on the anterior margin; the posterior margins of the first two pairs are equipped with five to seven teeth, but that of the third pair is toothless. The carpus is equipped with four spines. The propodus is equipped with three spines on the proximal part of the anterior margin and is furnished with three spinules along the inner margin. The dactylus is provided with an accessory claw on the inner margin. There are five spinules on the inner margin in common members of the genus. The dactylus of this species is diminished first four spinules except the distal one. The distal (fifth) spinule is greatly developed into an acuminate tooth, forming an accessory claw.

Fig. 2. *Galathea biunguiculata* sp. nov.
a: First peduncle of left antennule, ×40, b: Basal peduncles of left antenna, ×40, c: Third maxilliped of left side, ×40, d: Sternum of third maxilliped, ×25, e_1: Ambulatory leg of first pair ×12, e_2: The same of second pair, ×12, e_3: The same of third pair, ×12.

It has some resemblance to *Galathea gardineri* Laurie[1] in general appearance, but it is easily distinguished by the armature of the carapace and chelipeds. It is provided with five spines on the inner margin of the merus of the second ambulatory leg instead of no spine as in the case of *G. gardineri*. These two species differ distinctly from other members of the genus by the characteristic features of the dactylus of ambulatory legs and of the sternum of the cheliped, which is not described by Laurie.

Type: Ovigerous ♀, holotype, Cat. No. 100, Zoological Laboratory, Faculty of Agriculture, Kyushu University, from off Narsmau, Babldáob Island, Palau Is., July 14, 1939, collected by Dr. H. Ohshima and the author.

Habitat: Found on sandy and muddy bottoms 26 m deep, 134° 37′ 30″ E., 7° 44′ 20″ N.

Dimensions (in mm):

Length of carapace including rostrum	4.4
Breadth of carapace	3.3
Length of front	1.46
Breadth of front	1.46
Length of cheliped	6.0
Length of wrist	1.0
Length of palm	2.2
Length of movable finger	1.7

2. *Galathea tridentirostris* sp. nov.

Text-fig. 3–4.

The carapace including rostrum is slightly longer than broad; the upper surface is smooth and spinulose. The gastric region is more or less elevated, but is not separated by cervical groove, which is not distinct. The striations of the carapace are ten in number. The rostrum is rather broad, with three spines on each side. Anterolateral margins are strongly divergent, and posterior margins are parallel behind the second teeth.

The first peduncle of antennule is furnished with three spines. The inner spine, dorsally placed, is very small; an another inner spine, ventrally placed and the outer spine are rather broad and equal in length. The first peduncle of antenna is very broad, its

[1] Laurie, R. D. 1926 Trans. Linn. Soc., London, Ser. 2, Zool., **19** (1): 131–133, pl. 9, figs. 1–5.

inner distal angle very protruding, the anterior margin almost straight. The remaining peduncles are cylindrical, smooth and toothless. The anterior margin of the ischium of the third maxilliped is equipped with an acuminate tooth on both inner and outer angles. The merus is a little longer than broad; the inner margin is equipped with a broad tooth, tip pointed; the outer margin bears an acuminate tooth at the distal part. The carpus is almost smooth on the outer margin.

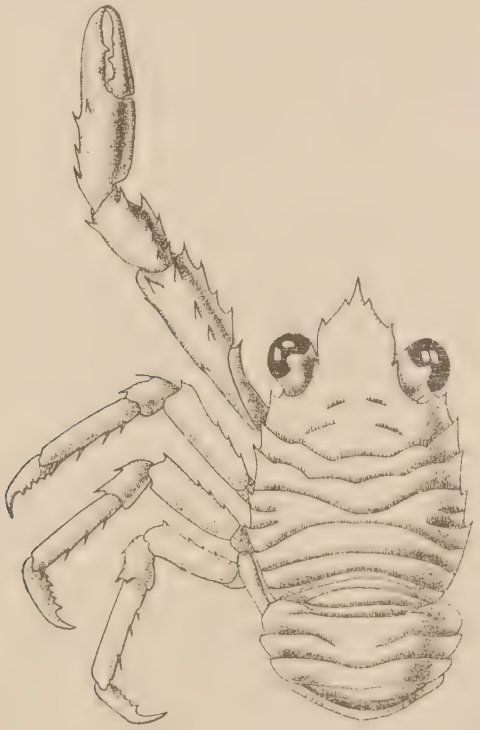

Fig. 3. *Galathea tridentirostris* sp. nov.
Holotype (♂) from Ishigaki Island, Ryukyu Islands, ×16.

The sternum of the third maxilliped is longer than broad; the lateral margins are almost parallel to each other; the anterior margin is triangular, with the tip rounded and furnished with a scale bearing long hairs at the center of the ventral surface.

The chelipeds are equal in length and twice as long as the carapace including rostrum in both sexes. The ischium bears a

spine on the inner distal angle. The arm is furnished with three rows of spines; the inner margin is furnished with four spines; the median row, running on the dorsal surface forms three enlarged spines; the outer margin forms two large spines. The wrist is a little shorter than the palm; the inner margin is furnished with four spines, the proximal one being rather small; in addition to them a row of three spines runs near the pronounced marginal spines; there is one spine at the outer distal end. The palm is almost smooth, but for the outer margin, which is equipped with four spines. The cutting margin of movable finger bears two teeth; the one is at the middle and the other is at the proximal end. The cutting margin of the immovable finger bears a tooth near the middle.

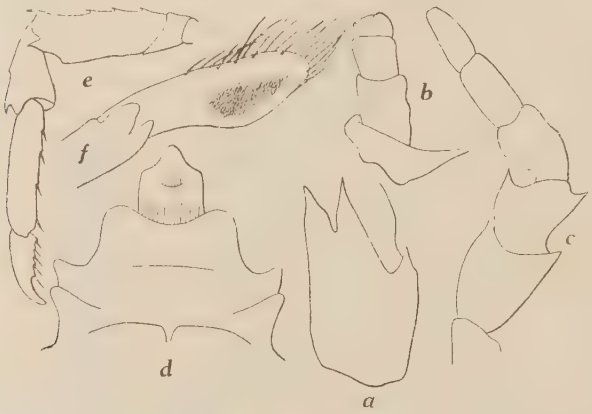

Fig. 4. *Galathea tridentirostris* sp. nov.
a: First peduncle of left antennule, ×40, b: Basal peduncles of right antenna, ×40, c: Third maxilliped of left side, ×40, d: Sternum of third maxilliped, ×25, e: Second ambulatory leg, ×25, f: Abdominal appendage of male, ×75.

The merus of the ambulatory legs is equipped with four spines on the upper margin; the carpus is furnished with one or two spines on upper margin, the distal one being enlarged; the propodus is equipped with four spinules on the posterior margin; the dactylus bears five spinules. Distal peduncle of male abdominal appendage is spoon-shaped.

This species is easily distinguished from the other members

of the genus by the broad and three-toothed rostrum and the shape of sternum of the third maxilliped.

Types: ♂, holotype; ovigerous ♀, allotype, Cat. No. 105, Zoological Laboratory, Faculty of Agriculture, Kyushu University; 4♂♂, 4 ovig. ♀♀, Cat. No. 106, Zool. Lab., Fac. Agr., Kyushu Univ.; from Ishigaki Island, Ryukyu Islands, May 22, 1940, collected by the author.

Habitat: Found on coral reef.

Dimensions (in mm):

	Holotype (♂)	Allotype (ovig. ♀)
Length of carapace including rostrum	3.3	3.5
Breadth of carapace	2.9	2.8
Length of rostrum	1.1	1.2
Breadth of rostrum	0.9	1.0
Length of cheliped	7.0	7.0
Length of arm	2.5	2.9
Length of wrist	1.2	1.0
Length of palm	1.5	1.4
Length of movable finger	1.2	1.2

3. *Galathea platycheles* sp. nov.

Text-fig. 5–6.

The carapace is of moderate length and more or less convex laterally. There is no spinule on the upper surface; cervical groove not indicated. The striation are 11 in number as in the Text-fig. 5. The rostrum is of moderate length, and provided with four prominent teeth. Lateral margins of the carapace are equipped with six teeth, excluding the tooth which forms the outer orbital angle; the second and sixth teeth are very small and faintly indicated.

The first peduncle of the antennule bears three spines on the distal margin. The second peduncle of the antenna is broader than long and equipped with a spinule on both inner and outer angles; the third peduncle is a little longer than broad; the fourth peduncle is small, broader than long. The ischium of the third maxilliped is furnished with an acuminate tooth on inner distal angle; the merus is smooth and furnished with single tooth which is very long, with the tip pointed; the carpus is smooth, provided with three protuberances with two or three long setae.

The sternum of the third maxilliped is smooth, much protruding upwards; the antero-lateral margin is almost straight; the median sinus is very small.

Fig. 5. *Galathea platycheles* sp. nov.
Holotype (♂) from Formosa, ×16.

The chelipeds are rather depressed and short, less than two times as long as the length of the carapace including rostrum in the male, and thickly pubescent, spinulose on the upper surface; the arm is slightly less than two-thirds the length of carapace includ-

ing rostrum, and equipped with five acuminate teeth: two of them are on inner margin, the distal one very long, which is accompanied by an accessory tooth at the base of inner side in the right arm only; one tooth is at the middle; the other two teeth are near the outer distal angle. In the right arm, moreover, one small tooth is present behind the outer marginal tooth. The wrist is shorter than the arm, the outer margin expanded and furnished with three teeth, distal one being prominent; inner margin is furnished with three long teeth, of which the middle one is largest; on the dorsal side there are two more teeth at the base of the inner marginal teeth; the palm is very broad and as long as arm, its outer margin being furnished with four or five teeth, two teeth accompanying the outer marginal teeth; while inner margin is equipped with three teeth which are small, but acuminate; the distal margin of the palm is furnished with an acuminate tooth; the movable finger is equipped with a strong tooth near the proximal end of the dorsal face, its cutting margin being equipped with teeth and provided with a tubercle at the base. The immovable finger also has a tubercle near the middle of the cutting margin.

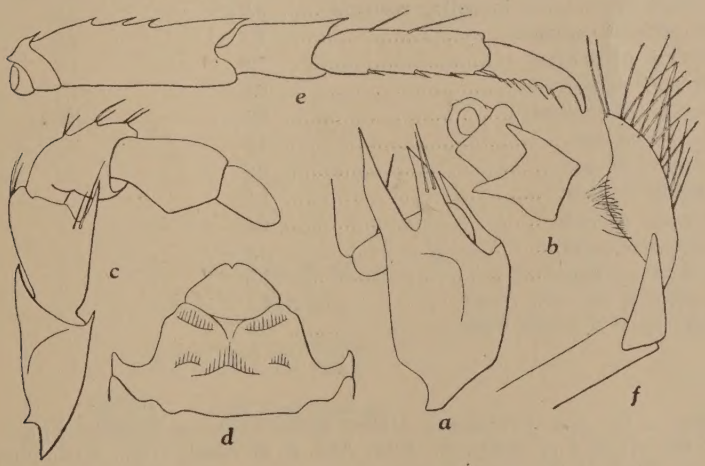

Fig. 6. *Galathea platycheles* sp. nov.
a: First peduncle of left antennule, ×40, b: Basal peduncles of right antenna, ×40, c: Third maxilliped of right side, ×40, d: Sternum of third maxilliped, ×25, e: Second ambulatory leg, ×25, f: Abdominal appendage of male, ×75.

The ambulatory legs are of moderate length; the merus is a little longer than the propodus, the upper margin is provided with three or four spines, the distal one being prominent; the carpus is about half the length of the merus, the upper distal end greatly protruding into an acuminate tooth; the propodus is somewhat hairy, the posterior margin is provided with three or four spinules; the dactylus is slightly longer than half the propodus, the posterior margin being furnished with four spinules.

The last joint of abdominal appendage in the male is much expanded at the middle, with the tip rounded.

It has some resemblance to *Galathea latirostris* Dana,[1] *Galathea providentia* Laurie[2] and *Galathea ternatensis* Melin,[3] in the armature of the rostrum, but it is easily distinguished by the armature of the merus of the third maxilliped.

Types: ♂, holotype, ovigerous ♀, allotype, Cat. No. 107, Zoological Laboratory, Faculty of Agriculture, Kyushu University; from Swô, Formosa, Nov. 4, 1932, collected by Dr. H. Ohshima.

Habitat: Found on coral reef.

Dimensions (in mm):

	Holotype (♂)	Allotype (ovig. ♀)
Length of carapace including rostrum	3.0	3.8
Breadth of carapace	2.3	3.0
Length of rostrum	1.0	1.3
Breadth of rostrum	0.9	1.1
Length of cheliped	5.8	missing
Length of arm	1.8	„
Breadth of arm	0.9	„
Length of wrist	1.6	„
Breadth of wrist	0.9	„
Length of palm	1.8	„
Breadth of palm	1.3	„
Length of movable finger	1.1	„
Breadth of movable finger	0.4	„

[1] Dana, J. D. 1952 Crustacea:—United States Exploring Expedition; and Atlas (1885): 480, pl. 30, f. 8. Balss, H. 1913 Abh. K. B. Akad. Wiss., Math.-phys. Kl., München, Suppl., **2** (9): 10-11, f. 9-10.

[2] Laurie, R. D. 1926 Trans. Linn. Soc., London, ser. 2, Zool., **19** (1): 125-128, pl. 8, f. 1-4.

[3] de Man, J. G. 1902 Abh. Senckenb. nat. Ges., Frankfurt a. M., **25** (3): 714-717. Melin, G. 1939 Kungl. Svenska Vetenskap-Akad. Handl., Tredje Ser., Stockholm, **18** (2): 67-72, f. 39-42.

九州大學農學部紀要
第10卷，第2册

昭和二十八年九月二十五日印刷
昭和二十八年九月三十日發行

編輯兼發行者　九州大學農學部

印刷者　九州大學内　堀川庄三

印刷所　九州大學印刷所